Cell. Mol. Life Sci. 65 (2008) 414 – 444
1420-682X/08/030414-31
DOI 10.1007/s00018-007-7305-6

Cellular and Molecular Life Sciences

Review

Histone chaperones: 30 years from isolation to elucidation of the mechanisms of nucleosome assembly and disassembly

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Received 5 July 2007; received after revision 8 September 2007; accepted 13 September 2007
Online First 22 October 2007

Abstract. Some three decades have passed since the discovery of nucleosomes in 1974 and the first isolation of a histone chaperone in 1978. While various types of histone chaperones have been isolated and functionally analyzed, the elementary processes of nucleosome assembly and disassembly have been less well characterized. Recently, the tertiary structure of a hetero-trimeric complex composed of the histone chaperone CIA/ASF1 and the histone H3-H4 dimer was determined, and this complex was proposed to be an intermediate in nucleosome assembly and disassembly reactions. In addition, CIA alone was biochemically shown to dissociate the histone $(H3\text{-}H4)_2$ tetramer into two histone H3-H4 dimers. This activity suggested that CIA regulates the semi-conservative replication of nucleosomes. Here, we provide an overview of prominent histone chaperones with the goal of elucidating the mechanisms that preserve and modify epigenetic information. We also discuss the reactions involved in nucleosome assembly and disassembly.

Keywords. Histones, chaperones, nucleosome assembly, nucleosome disassembly, mechanisms, overview.

Introduction

The preservation and expression of genetic information by processes such as transcription, DNA replication, repair, and recombination play fundamental roles in biological phenomena like cell proliferation, differentiation, tumorigenesis, senescence, and death. In eukaryotes, genomic DNA forms chromatin structures with histones, which are evolutionarily highly conserved basic proteins, and various histone-associated chromatin factors. The structure of chromatin blocks access of various enzymes and factors that facilitate DNA-mediated reactions on the DNA. Therefore, the maintenance and regulation of chromatin structure are essential for eukaryotic gene regulation.

Chromatin consists of a repetitive nucleosomal structure. The nucleosome is composed of a core histone octamer formed by two molecules each of the four core histones, namely H2A, H2B, H3, and H4, and 146 base pairs of DNA wrapped around the histone octamer. Histones contain two structural and functional domains, an N- or C-terminal tail region and the core domain. The N- or C-terminal tail region is extensively modified, either transiently or continuously, by histone modification enzymes such as histone acetyltransferases, histone methyltransferases, and

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histone kinases. These modifications influence individual gene expression as well as the formation of functional chromosomal domains. The core domain defines a scaffold for the nucleosome structure. Despite the distinct primary structures, the core domains of the four core histones adopt essentially the same fold, the histone fold. This fold is utilized for heterodimer formation and is shared with various eukaryotic histone-associated chromatin factors, such as some TFIID subunits, and with some archaeal DNA-associated proteins. These tail regions and the core domains form the molecular bases of chromatin's structural and functional features.

Two types of components are important for defining chromatin features: DNA binding factors that regulate specific gene expression, and histone-associated chromatin factors that alter nucleosome structure and function. The latter type includes histone modification enzymes, ATP-dependent nucleosome remodeling complexes, and nucleosome assembly/disassembly factors, also referred to as histone chaperones. Despite their large numbers, most of these enzymes and factors, with the exception of the histone chaperones, can be classified into relatively small numbers of protein families. Therefore, determining their structure-function relationships has been straightforward. In contrast, the primary structures of the ten kinds of isolated histone chaperones are highly diverse. In addition, a unified understanding of their structure-function relationships has not yet been achieved due to limited information on their tertiary structures. A recent expansion in histone chaperone research based on structure-function analyses has revealed a number of similarities and differences among histone chaperones and has shed light on the mechanisms of nucleosome assembly and disassembly.

In this review, we discuss the structural and functional features of histone chaperones that have been identified and characterized to date. We also discuss nucleosome assembly and disassembly activities that are relevant to transcription, DNA replication, repair, and recombination.

Histone chaperones

Nucleosomes can be reconstituted from DNA and histones by salt gradient dialysis [1–3]. However, these mixtures are aggregated and precipitated at physiological ionic strength. Laskey et al. [4] constructed a cell-free nucleosome assembly system at physiological ionic strength with *Xenopus* egg extracts and used this system to isolate nucleoplasmin as a nucleosome assembly factor that does not require low-molecular-weight cofactors, such as ATP. They termed this factor a molecular chaperone (in this case, a "histone chaperone") because it prevents nonspecific interactions between DNA and histones and promotes specific interactions that lead to nucleosome assembly [5, 6].

Subsequent work showed that the slow addition of histone made it possible to assemble nucleosomes without the histone chaperone at near physiological ionic strength. Under these conditions, however, nucleosome assembly could be stimulated by the addition of other chromatin components (*e.g.*, the linker histone H1 and the non-histone protein HMG [7, 8]) and acidic polymers (*e.g.*, acidic polypeptide [9, 10], poly (L-malate) [11], and RNA [12]). These studies resulted in the isolation of other histone chaperones with different primary structures and demonstrated that there are various types of histone chaperones [13].

In this review, we define a histone chaperone as a factor that has (i) a histone binding activity and (ii) a histone-dependent, ATP-independent nucleosome assembly activity, on the basis of the properties of nucleoplasmin, the first histone chaperone discovered. Notably, most histone chaperones have a preference for binding either histones H3-H4 or H2A-H2B. Despite numerous attempts, there is currently no unified view of the mechanism by which histone chaperones promote nucleosome assembly. In most studies, nucleosome formation is monitored by a supercoiling assay [4, 14, 15] that detects the superhelical structure of circular DNA created by nucleosome assembly. In addition, the length of DNA wrapped around the histone octamer can be determined by digestion of nucleosome-associated circular DNA with an endogenous nuclease such as micrococcal nuclease (MNase) [2, 3, 16, 17]. The formation of a chromatin-like repeating unit can also be confirmed by X-ray diffraction [1] or by electron microscopy [3, 18].

Biochemical analyses have revealed that histone chaperones interact not only with histone proteins but also with numerous other chromatin factors. Isolation of the histone H3.1 and H3.3 complexes using epitope-tagged histones [19] showed that histone chaperones are included in the histone H3.1 and H3.3 complexes, in addition to other chromatin factors. Similarly, isolation of the H2AZ complex revealed the presence of several chromatin factors in the complex [20]. These interactions between histone chaperones and chromatin factors are important for the biochemical and biological functions of histone chaperones.

Here, we summarize the roles of ten types of histone chaperones, which have been identified on the basis of the functional criteria discussed above. We also

Table 1. A list of histone chaperones.

Chaperone	Year (activity)	Family and homologues	Homology	Crystal structure	Histone selectivity	Interactions	Related function
Nucleoplasmin	1978 [5]	NPM1/numatrin/B23(h,m,r), No38/ nucleophosmin(x) NPM2(h,m,r), nucleoplasmin(x) NPM3(h,m), NO29(x) NLP(d)	34 % : drosophila – human (dNLP – hNPM3)	nucleoplasmin [34] NO38 [35] dNLP [36]	H2A/H2B	SP(x) [21]	Storage of H2A/H2B pools in Xenopus oocytes [5, 39] Sperm-chromatin decondensation [28] Ribosome biogenesis [27] Apoptotic chromatin condensation [42]
NAP1	1983 [96]	NAP1/NAP1L(h,m,r), NAP1(d,x,Ce,Sp,Sc)	36 % : yeast – human	yNAP1 [106]	H2A/H2B	H1(x) [113], B4(x) [114]	Histone H2A variant exchange [20, 111, 124]
		NAP1L2(h,m,r), NAP1L3(h,m,r), NAP1L4/ NAP-2(h,m,r), NAP1L5(h,m)	(yNAP1 – hNAP1)			E2(h) [126], Tbr-1(h) [110]	Nucleosome sliding [111]
		TSPY(h,m,r), TSPYL1(h,m,r), CINAP/ TSPYL2/DENTT(h,m)					Linker histone deposition [113, 114]
		TSPYL3(h,m), TSPYL4(h,m,r), TSPYL5(h), TSPYL6(h)					Histone shuttling [117, 118]
							Organ-specific expression [125]
							Transcriptional regulation [110, 126]
N1/N2	1985 [39]	NASP(h,m,r), N1/N2(x)	41 % : xenopus – human	–	H3/H4	Hat1(Sc) [55, 56], H1(h) [59],	Storage of H3/H4 pools in Xenopus oocytes [38, 39]
		Hif1(Sc)	(xN1 – hNASP)			HSP90(h) [64]	Linker histone deposition [62]
			18 % : yeast – human				Linker histone transportation [63]
			(yHif1 – hNASP)				
CAF-1	1989 [154]	p150(h), p180(d), Cac1/Rlf2(Sc)	31 % : yeast – human	–	H3/H4	PCNA(h) [163], HP1(h) [167],	DNA replication [153]
			(yCac1 – p150(h))			Sas2(y) [243], Sas4(y) [243]	DNA repair [159, 178]
		p60(h), p105(d), Cac2(Sc)	34 % : yeast – human			CIA-I(h) [173], CIA-II(h) [173],	Telomere Silencing [159, 160]
			(yCac2 – p60(h))			CIA(d, y) [158, 172]	Cell cycle regulation [166]
		p48(h), p55(d), Cac3/Msi1(Sc)	30 % : yeast – human			HAT1 complex(h) [175],	
			(yCac3 – p48(h))			HDAC1 complex(d) [157],	
						ESC-E(Z) complex(d) [180],	
						NURF complex(d) [175]	

Table 1 *(Continued)*

Chaperone	Year (activity)	Family and homologues	Homology	Crystal structure	Histone selectivity	Interactions	Related function
Spt6	1996 [79]	SUPT6H(h,m), Spt6(d,Sp), EMB-5(Ce), Spt6/Cre2/Ssn20(Sc)	25 % : yeast – human	–	H3/H4	Spt5(Sc) [87], Iws1(Sc) [88],	Transcription elongation [86, 328]
			(ySpt6 – hSpt6)			Rpb1(Sc) [88]	Transcriptional repression [70–72, 83]
						exosome complex(d) [95]	mRNA processing [95]
							mRNA 3′-end formation [94]
TAF-Iβ/SET	1996 [107]	TAF-Iβ/SET/PHAPII/INHAT/IGAAD/ StF-IT/I2PP2A(h), SET(m,r,x,d)	22 % : yeast – human	hTAF-Iβ [134]	H3/H4	KLF5(h) [140], Sp1(h) [141],	Cancer regulation [127, 145, 152]
		Spr-2(Ce)	(yVps75 – hTAF-Iβ)			ERα(h) [142],	Sperm-chromatin decondensation [133]
		Vps75(Sc)				progesterone receptor B(h) [142],	DNA replication [128]
						thyroid receptor β(h) [142],	Inhibition of the histone acetyltransferase activity [135]
						RXRα(h) [142], Fe65(h) [144],	Caspase-independent apoptosis [146–149]
						Rtt109(Sc) [131]	Cell cycle regulation [150, 151]
							Transcriptional regulation [107, 129, 140–144]
CIA/ASF1	1999 [184], 2000 [221]	CIA-I/ASF1a(h,m,x), CIA/ASF1(d,Sc), Cia1(Sp)	58 % : yeast – human	yCIA/Asf1 [216, 227]	H3/H4	CCG1(h) [226], Bdf1(y) [226],	Transcriptional regulation [185, 226, 231]
		CIA-II/ASF1b(h,m,r,x)	(yCIA – hCIA-I)	hCIA-I/ASF1a [222]		Bdf2(y) [226], HIRA(h) [171, 216, 217],	DNA replication [184]
				hCIA-I-H3-H4 [224]		Hir1(y) [185, 213], Hir2(y) [185],	DNA repair [184, 225, 240]
				yCIA-H3-H4 [223]		CAF-I p60(h) [173], CAF-I p105(d) [158],	DNA recombination [241]
				hCIA-HIRA peptide [171]		Cac2(y) [172], RFC complex(h) [235],	Gene silencing/anti-silencing [184, 185, 213, 225, 242, 243]
						Tlk1(h) [229], Tlk2(h) [229], Tlk(d) [245],	Regulation of HAT activity [253–255]
						Sas4(y) [242, 243], Sas2(y) [243],	Cell death [247]
						Sas5(y) [243], Rad53(y) [237–239]	Cell senescence [217]

Table 1 *(Continued)*

Chaperone	Year (activity)	Family and homologues	Homology	Crystal structure	Histone selectivity	Interactions	Related function
HIRA	2002 [195]	HIRA(h,m,x,d)/TUPLE1(h), Hip1(Sp), Slm9(Sp), Hir1(Sc), Hir2/Spt1(Sc)	29 % : yeast – human	hCIA-HIRA peptide [171]	H3/H4	Hir3(y) [202, 203], Hpc2(y) [202, 203],	Sperm-chromatin decondensation [219]
		Tup1(Sc)	(yHir1 – hHIRA)			CIA-I(h) [171, 216, 217], CIA(y) [185, 213]	Histone H3.3 deposition [219]
		E(spl)(d)	22 % : yeast – human			CAF-I p48(h) [212], HDAC2(h) [212],	Transcriptional regulation [191, 192, 198, 200, 211]
			(yHir2 – hHIRA)			Pax3(h) [198], Pax7(h) [198],	Gene silencing [169, 185, 213, 215]
						HIRIP3(h) [197], HIRIP5(h,m) [329]	Cell cycle regulation [209, 210]
							Cell senescence [217]
FKBP	2004 [259]	Fpr3(Sc), SpFkbp39(Sp)	36 % : yeast – human	–	H3/H4	FK506, FK520, rapamycin	rDNA silencing [259]
		Fpr4(Sc)	(yFpr3 – hNCL)			S24(Sc) [263]	Regulation of histone methylation [268]
			*N-terminus only				Cell cycle regulation? [273]
JDP2	2006 [279]	JDP2(h,m,r)	96 % : mouse – human	–	H3/H4	c-jun(r) [277], JunB(r) [277], JunD(r) [277],	Transcriptional regulation [277, 278, 282, 284]
			(mJDP2 – hJDP2)			ATF-2(m) [278], C/EBPγ(r) [283],	Tumorigenesis [288, 289]
						HDAC3(m) [284]	Inhibition of HAT activity [279]
						progesterone receptor A(h) [282]	Cell differentiation [284, 286, 287]
							Cell death [290, 291]

(h) Human; (m) Mouse; (r) Rat; (x) Xenopus; (d) Drosophila; (Ce) *C. elegans*; (Sp) *S. pombe;* (Sc) *S. cerevisiae.*

suggest directions for future examinations of nucleosome assembly and disassembly mediated by histone chaperones.

Nucleoplasmin/nucleophosmin (NPM)

The histone chaperone nucleoplasmin was identified in *Xenopus* egg extracts as a factor that binds histones and loads them onto DNA [5, 6]. The nucleoplasmin/nucleophosmin (NPM) family is comprised of four groups: NPM1 (nucleophosmin, B23, numatrin, NO38), NPM2 (nucleoplasmin), NPM3, and NLP (NPM-like proteins), which are defined on the basis of sequence homology [21].

NPM1 was identified as a phosphoprotein that is highly expressed in the nucleolus [22, 23]. NPM1, which was initially thought to be important for ribosome assembly, has since been found to have roles in many important cellular processes. The nucleosome assembly activity of NPM1 was identified later [24]. NPM2 was identified as a factor that mediates the assembly of nucleosomes [5]. After this activity was identified, NPM2 was also implicated in sperm chromatin decondensation and in the activation of transcription factors that interact with nucleosomes [21]. *Npm3* was discovered as a gene located near the proto-oncogene *fgf8* during studies to identify and isolate proto-oncogenes that are activated by mouse mammary tumor virus (MMTV) proviral insertions in tumors from infected Wnt-1 transgenic mice [25, 26]. NPM3 has been implicated in the regulation of NPM1 activity relevant to ribosomal RNA synthesis [27]. In addition, similar to NPM2, NPM3 may be involved in the regulation of sperm chromatin decondensation [28]. The NLP are invertebrate homologues of nucleoplasmin. The best-characterized NLP is the *Drosophila* NLP (dNLP) [29]. dNLP can bind histones and decondense sperm chromatin. It is intriguing that dNLP requires ATP for its nucleosome assembly activity [29].

NPM family proteins have two distinct domains: a conserved N-terminal core domain, which includes an acidic region A1, and a divergent C-terminal tail domain, which includes one or two acidic regions (A2 and/or A3) (Fig. 1a). The N-terminal core domain is responsible for the oligomerization of subunits and for histone chaperone activity. The C-terminal tail domain, which varies in length among the family members, contains some functional motifs. The A1 and A2/A3 regions are suggested to play a role in sperm decondensation and histone binding, respectively [30, 31]. NPM1, NPM2 and NPM3 have classical bipartite nuclear localization signals (NLS) with the consensus sequence $KRX_{10}KKK$, where X can be any amino acid [32]. Additional motifs that are exclusively found in NPM1 proteins include a putative nuclear export signal (NES) and a C-terminal extension containing both a putative nucleolar localization signal (NoLS) and a domain for nucleic acid binding and RNA cleavage (Fig. 1a) [33].

The crystal structures of the *Xenopus* NPM1 (NO38), NPM2 (nucleoplasmin), and the dNLP N-terminal core domains have been determined (Fig. 1b) [34–36]. These structures suggested that xNPM1 and xNPM2 are decamers and that dNLP is a pentamer. The subunits of these histone chaperones adopt essentially the same fold: an eight-stranded β-barrel structure. Biochemical and modeling studies suggest that the decameric histone chaperones xNPM1 and xNPM2 bind five histone octamers. Two subunits in the decameric chaperone appear to interact with one histone octamer. Although dNLP is a pentamer, the functional unit of this chaperone may be a decamer similar to those formed by xNPM1 and xNPM2 [35]. In addition, heterogeneous complexes of different NPM family members may allow for functional modulation [27, 37].

Biochemical analysis has shown that the N-terminal core domain of NPM1 interacts with the histone $(H3\text{-}H4)_2$ tetramer and has no nucleosome assembly activity [31]. Since full-length NPM1 has nucleosome assembly activity, the C-terminal tail domain may bind the histone H2A-H2B dimer and may be required for nucleosome assembly activity [31]. Although NPM1 can bind both histone $(H3\text{-}H4)_2$ tetramer and H2A-H2B dimers, NPM1 has been reported to bind the histone $(H3\text{-}H4)_2$ tetramer preferentially [24, 31, 36], whereas NPM2 binds histones H2A-H2B preferentially [34]. In *Xenopus* oocytes, histones H2A-H2B and H3-H4 form complexes with NPM2 and N1/N2 (see below), respectively [38, 39]. Although each of these histone chaperones can independently assemble nucleosomes *in vitro*, assembly is more efficient when both are present [40].

Phosphorylated NPM family proteins play a role in chromatin condensation/decondensation in collaboration with other histone chaperones. Chromatin structures in sperm are highly condensed by sperm-specific proteins (SPs), and decondensation is necessary for organization of the diploid genome. A mechanism of sperm chromatin decondensation has been proposed [21]. During oocyte maturation, NPM2 becomes phosphorylated [41]. Histone H2A-H2B dimers are stored in oocytes through their interactions with the lateral face of the NPM2 decamer. When hyperphosphorylated NPM2 associated with histone H2A-H2B dimers comes into contact with the highly compacted sperm chromatin following fertilization, SPs dissociate from the sperm chromatin and bind to the distal face of the decamer through electrostatic interactions. SP-NPM2 interactions may cause a conformational change in the

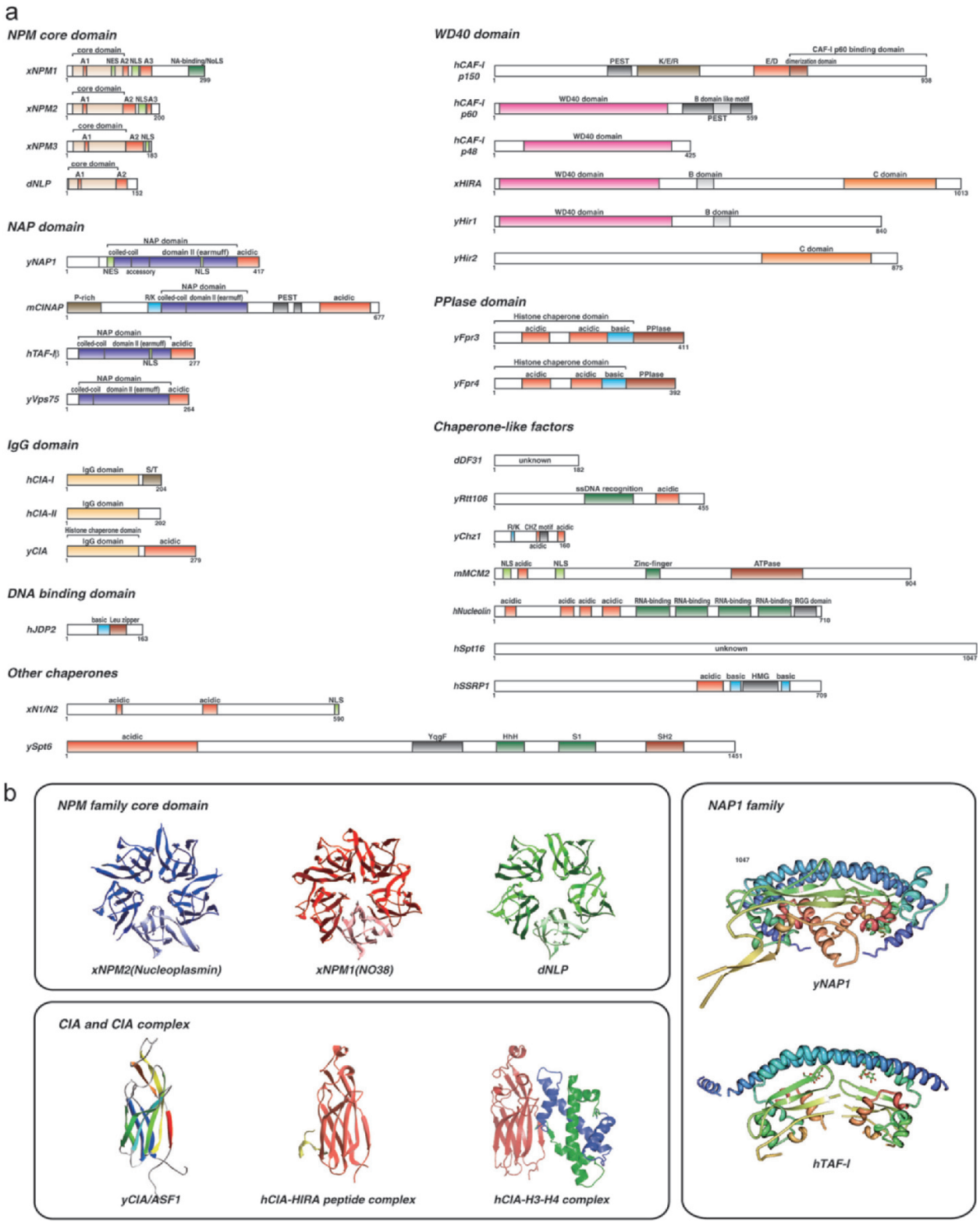

Figure 1. Structural features of histone chaperones. (*a*) Domain structure of members of the histone chaperone families. Domain and motifs are represented in black (PEST, CHZ, RGG, YqgF, HMG domains), dark brown (K/E/R-rich, S/T-rich, or P-rich domains), light brown (NPM core domain), dark red (ATPase, PPIase, SH2, dimerization, or Leu zipper domains), light red (acidic domain), orange (C domain), yellow (IgG domain), light green (NLS or NES), dark green (HhH, S1, zinc-finger, RNA-binding, NA-binding, or ssDNA-recognition domains), cyan (basic domain), dark blue (NAP domain), pink (WD40 domain), and gray (B domain). (*b*) Crystal structures of histone chaperones. The PDB codes for the structures are xNPM2(1K5J), xNPM1(1XBQ), dNLP(1NLQ), yCIA/ASF1(1WG3), hCIA-HIRA peptide complex(2I32), hCIA-H3-H4 complex(2IO5), yNAP1(2AYU), and hTAF-Iβ(2E50).

NPM2 decamer, resulting in the release of the bound histone H2A-H2B dimers for delivery to sperm DNA with histone $(H3\text{-}H4)_2$ tetramers, thus allowing nucleosomes to form. Following the exchange of histone H2A-H2B dimers for SPs, NAP1 adds the linker histone B4 and chromatin in the paternal pronucleus, and thus resembles that of the maternal pronucleus. Following replication of the paternal DNA, N1/N2 would be needed to deposit histone $(H3\text{-}H4)_2$ tetramers before the nucleoplasmin adds histone H2A-H2B dimers.

In contrast to its role in sperm chromatin decondensation, *Xenopus* NPM2 also contributes to apoptotic chromatin condensation, and this reaction is regulated by NPM2 phosphorylation [42]. The phosphorylation of NPM1 also regulates its activity. NPM1 phosphorylated at Thr199 by CDK2/cyclin E is involved in centrosome duplication [43, 44] and the repression of pre-mRNA splicing [45]. NPM family proteins undergo other post-translational modifications in addition to phosphorylation. NPM1 is acetylated by the histone acetyltransferase p300, resulting in an increase in the transcription of a p53-responsive synthetic reporter gene [31]. Sumoylation on both Lys230 and Lys260 residues of NPM1 regulates its subcellular localization, cell proliferation, and survival activities [46]. The components of a neuronal gene repressor complex that includes NPM1 are modulated by poly(ADP-ribosyl)ation, which allows for the dissociation of poly(ADP-ribosyl)ated components, including NPM1, from the complex when it is bound to a promoter [47]. Both NPM2 and NAP1 have glutamylation motifs in their C-terminal acidic regions [48, 49].

A unique characteristic of NPM histone chaperones is decamer (or pentamer) formation through their N-terminal core domains. The multimeric structure of NPM family proteins may be advantageous for storing large amounts of histones in the egg. Since the tertiary structures of the individual NPM family proteins are similar to one another, some of these proteins may be able to form hetero-decamers. Hetero-decamerization of NPM family proteins may modulate their functions. The structural significance of the nucleosome assembly activity of NPM family proteins remains to be resolved. Future studies of these proteins will include structural analysis of NPM-histone complexes and biochemical analysis of the cooperation between NPM and N1/N2 in nucleosome assembly.

N1/N2

In 1975, N1 and N2 (M_r ~105 and ~110 kDa) were isolated as proteins that accumulate in the nucleoplasm when *Xenopus* nuclear extracts were injected into *Xenopus* eggs [50, 51]. Subsequently, Kleinschmidt and colleagues showed that N1 and N2 form a complex with histones H3 and H4 and possess histone chaperone activity [38]. cDNA clones of N1 and N2 were isolated on the basis of the amino acid sequence of the peptide fragments that were obtained using antibodies specific for N1 and N2 [52]. The DNA sequences of N1 and N2 are identical, and the differences in biochemical and immunochemical properties have not been explained. These proteins were therefore designated N1/N2. Homologues of N1/N2 have been found in other species. Mammalian NASP (nuclear autoantigenic sperm protein) [53, 54] and yeast Hif1 (Hat1 interacting factor 1) [55, 56] have sequence similarities to N1/N2. N1/N2 and NASP possess two acidic histone binding clusters in the N-terminal region and a nuclear localization signal in the C-terminal region (Fig. 1a) [52, 57–59]. There are two types of NASP, a testis-specific form (tNASP) and a somatic form (sNASP) that is a splicing isoform of tNASP [54, 59]. This suggests that NASP is involved in the formation of chromosomes from both somatic and sperm DNA.

N1/N2 preferentially interacts with histones H3 and H4 in *Xenopus* egg extracts and shows *in vitro* nucleosome assembly activity [39, 60]. Analyses of the nucleosome assembly activities of N1/N2- and/or nucleoplasmin-depleted *Xenopus* egg extracts, as well as the activities of the purified proteins, indicate that N1/N2 and nucleoplasmin coordinately deposit histones onto DNA in a two-step manner [40, 61]. N1/N2 deposits histones H3 and H4 onto DNA to form an intermediate complex. Nucleoplasmin then loads histones H2A and H2B onto this complex to form a nucleosome. Human NASP is included in the histone H3.1 and histone H3.3 complexes in HeLa cells [19], suggesting that NASP may interact with the histone H3-H4 complex and may have a nucleosome assembly activity similar to N1/N2. In contrast, yeast Hif1 requires a cytosolic extract for nucleosome assembly, and this reaction buffer includes ATP [56]. This indicates that the mechanism of Hif1 activity in nucleosome assembly differs from that of N1/N2.

NASP can bind the linker histone H1 [59] and competes with DNA for histone H1 [62]. In addition, NASP interacts with the heat-shock protein HSP90 and activates its ATPase activity [63]. The physical interactions between NASP, linker histone H1, and HSP90 appear to function as follows. The amount of linker histone H1 that binds to NASP and is transported into the nucleus increases in an ATP-dependent manner [63], suggesting that an ATP-dependent molecular chaperone activity of HSP90 helps to fold NASP, thereby allowing it to interact with linker histone H1. Since NASP interacts with HSP90 [64] and stimulates its ATPase activity [63], NASP activates HSP90 or vice versa. The DNA binding factors

E2F and Sp1 bind to the *NASP* promoter [65–67], suggesting that they regulate the increase in *NASP* gene expression in the S phase [59]. During the cell cycle, NASP shuttles between the nucleus and cytoplasm [62, 68, 69]. These results all indicate that NASP may transport linker histone H1 into the nucleus in the presence of HSP90 in the S phase and deposit this histone onto replicated DNA. This hypothesis is supported by the finding that knockdown or overexpression of NASP inhibits S-phase progression [62, 69]. Since NASP has sequence similarity to N1/N2 and is included in the histone H3.1 and histone H3.3 complexes [19], it may be a factor that links nucleosome assembly and deposition of the linker histone H1.

Spt6

In 1984, Winston and colleagues isolated *spt6* as a mutation that suppresses the Ty element-mediated repression of the *HIS4* gene [70]. Later, the *SPT6* gene was independently isolated as the *cre2* (catabolite repressor element 2) [71] and *ssn20* (suppressor of *snf2* 20) [72] mutations, which suppress the repression of the *ADH2* and *SUC2* genes, respectively [73–75]. Furthermore, Spt6 has been identified in yeast, *Caenorhabditis elegans,* and mammals [76–78], indicating that it is an evolutionarily conserved factor. In 1996, Spt6 was demonstrated to have both histone binding and histone chaperone activities [79]. Spt6 binds histones H3-H4 preferentially [79].

Spt6 is a 170-kDa nuclear protein whose N-terminal region abounds with acidic amino acid residues and whose C-terminal region contains an SH2 domain, a YqgF-homologous domain, tandem HhH motifs, and an S1-like domain (Fig. 1a) [76, 80–82]. The YqgF-homologous domain, HhH motifs, and S1-like domain are likely to possess nuclease, DNA binding, and RNA binding activities, respectively. The primary structures of the YqgF-homologous and S1-like domains are similar to that of the bacterial factor Tex. The Tex protein is hypothesized to regulate transcriptional process [82]. Spt6 is involved in the transcriptional repression of several genes, such as *HTA1-HTB1* (which encode histones), *HIS4*, *ADH2*, and *SUC2* [70–74, 83]. Since Spt6 localizes to promoters [84] and is involved in chromatin assembly on promoter and ORF regions [79, 84, 85], its activities probably lead to transcriptional repression.

Spt6 is also capable of stimulating transcription elongation, as several lines of evidence indicate. The first such line is that Spt6 can stimulate transcription elongation in concert with the DRB-sensitivity-inducing factor (DSIF), which is composed of Spt4 and Spt5 [86]. Spt6 and DSIF may interact physically, since Spt5 can interact with Spt6 [87]. Second, Spt6 can be copurified with the C-terminal domain (CTD) of RNA polymerase II as well as with other transcription elongation factors/complexes, including S-II, DSIF, FACT, P-TEFb, TFIIF, and Paf1/Rtf1 [88]. Third, Spt6, DSIF, and FACT inducibly colocalize on promoter and ORF regions with cyclin T, a kinase subunit of P-TEFb, and RNA polymerase II with phosphorylated CTD, which is an actively elongating form [89–91]. However, RNA polymerase II with unphosphorylated CTD, which is inactive for elongation, does not induce this colocalization [89–91]. Phosphorylated CTD is specifically recognized by the SH2 domain of Spt6 [92]. Fourth, genetic analyses have supported the transcription elongation activity of SPT6. Deletion of *SPT6* is lethal [73, 74], and mutation of *SPT6* yields a 6-azauracil-sensitive phenotype indicative of transcription elongation defects [86]. Moreover, the *spt6 S-II* double mutant shows a temperature-sensitive phenotype, similar to that seen for the *spt4 S-II* and *spt5 S-II* double mutants [86]. These results all suggest that Spt6 is involved in nucleosome assembly through its histone chaperone activity during elongation.

In addition, Spt6 was found to be involved in the synthesis, processing, and export of mRNA. Spt6 comigrates with DSIF, FACT, and RNA polymerase II to polyadenylation sites on the *ADH1*, *PMA1*, and *PYK1* genes during transcription elongation [93]. Spt6- or Spt4-defective mutant produces lower levels of the *GAL10-GAL7* fusion transcript, suggesting that Spt6 can affect the 3′-end formation of mRNA [94]. Spt6 coimmunoprecipitates with RNA-processing exosomes, which comigrate along with the *hsp70* and *hsp26* genes during transcription elongation [95]. Iws1, a direct binding partner of Spt6 [88], also interacts with export factors of mRNA [92]. These results suggest that Spt6 plays an important role in mRNA synthesis, processing, and export.

NAP1

Nucleosome assembly protein 1 (NAP1) was isolated from both human HeLa S3 and mouse FM3A cells as a protein that facilitates the assembly of nucleosome-like structures *in vitro* [96, 97]. NAP1 has been used as a reagent for defined *in vitro* chromatin assembly systems [98]. Homologues of NAP1 have been found in diverse eukaryotic organisms [99–104] and there are several NAP1 family members, as described below. NAP1-like proteins (NAP1L1–NAP1L5) have been identified by their sequence homology to NAP1. Template activating factor I (TAF-I), which has been implicated in cancer, is a well-known NAP1-like factor. The calcium/calmodulin-dependent serine protein kinase-interacting nucleosome assembly protein (CINAP), which is abundant in neuronal cells, and the testis-specific protein Y-encoded (TSPY),

which is evolutionarily conserved on the mammalian Y chromosome, are also NAP1 family proteins. In this section, we discuss NAP1 family proteins except TAF-I. TAF-I is described in the next section because it has been extensively studied.

NAP1 family proteins are composed of N- and C-terminal variable regions and a central highly conserved NAP domain containing approximately 300 amino acid residues (Fig. 1a). The NAP domain is necessary and sufficient for both histone binding and nucleosome assembly activities [105], and is also important for dimerization of NAP1 [106]. Other NAP1 family members may also possess these activities because their NAP domains share a sequence homology [107–110]. The C-terminal region, which is highly acidic, is required for transient removal of histone H2A-H2B dimers, facilitating the nucleosome sliding [111]. In many eukaryotes, NAP1 binds histone H2A-H2B preferentially *in vivo* [96, 102, 112]. The crystal structure of NAP1 reveals that it has a dome-shaped architecture defined by a long dimerization helix and an α-β domain in each monomer (Fig. 1b) [106]. However, the mechanism underlying NAP1-mediated nucleosome assembly remains elusive because the structure of the NAP1-histone complex has not been solved.

NAP1 is a nucleosome assembly factor not only for the core histones (H2A, H2B, H3, and H4) but also for the linker histones (H1 and B4). NAP1 has therefore been called a linker histone chaperone [113, 114]. NAP1 relaxes the spacing between nucleosomes by removing the linker histone H1 from the chromatin fiber [113]. Conversely, *Xenopus* NAP1 introduces the oocyte-specific linker histone B4 onto the chromatin after fertilization [114]. The linker histones H1 and B4 differ drastically in both the length and net charge of their C-terminal tail domains. The reduced basicity of the linker histone B4 compared with that of H1 in the long C-terminal tail domain might contribute to the reduced interaction between adjacent nucleosomes [115]. Thus, the deposition of linker histone B4 onto the chromatin by NAP1 may play an important role in early embryonic development when rapid rounds of DNA replication are taking place. This functional role is consistent with the embryonic lethality of a NAP1 knockout mutation in *Drosophila* [116].

NAP1 is involved in histone shuttling between the cytoplasm and nucleus. *Drosophila* NAP1 (dNAP1) changes its localization in a cell-cycle-dependent manner. In embryonic cells, dNAP1 is present in the nucleus in the S phase but is found predominantly in the cytoplasm in the G2 phase [102]. Phosphorylation of both dNAP1 and human NAP1L4 by casein kinase 2 causes them to localize to the nucleus [117, 118]. Yeast NAP1 (yNAP1) mediates an interaction between the histone H2A-H2B dimer and karyopherin, a nuclear import receptor [119]. Structural analysis of yNAP1 supports these phenomena [106]. The nuclear export of yNAP1 is dependent on its NES-like sequence [120]. A NES-like sequence located at the tip of the dimerization helix of yNAP1 is masked by the accessory domain, which is composed of helix α3 and flanking loop regions of the adjacent subunit and is linked to the α2 dimerization domain [106]. Therefore, the nuclear localization of NAP1 may be regulated by the masking and unmasking of the NES-like sequence [106]. Since the accessory domain is highly variable, different NAP1 family proteins may exhibit differences in the efficiency of nuclear export. NAP1 also has an NLS motif in a β hairpin. The NLS motif is a typical one found in other known nuclear proteins [121–123]. These observations provide evidence for the involvement of NAP1 in histone shuttling between the cytoplasm and nucleus.

In addition, yNAP1 and human NAP1 (hNAP1) are involved in exchange reactions with histone H2A variants [20, 111, 124]. yNAP1 is a subunit of the H2AZ complex, in which yNAP1 seems to be involved in the exchange of the canonical histone H2A with its variant counterpart H2AZ [20]. hNAP1 may also participate in exchanges between histone H2A and the H2ABbd variant [124]. These observations suggest that the intrinsic histone exchange activity of NAP1 is nonspecific for histone H2A variants and that specificity is conferred in the context of a larger macromolecular complex, such as the SWR1 complex.

The multi-functional histone chaperone NAP1 is conserved from yeast to human and defines a diverse family in higher eukaryotes. NAP1 family proteins are expressed in an organ-specific manner [125]. For example, members of the NAP1L subfamily and CINAP are expressed in the brain, while TSPY is testis specific. A better understanding of the relationship between the diversity of NAP1 family proteins and their organic specificity is awaited. In addition, NAP1 family members were shown to interact with various gene-specific transcription factors including the transcriptional activator E2 [126] and the T-box transcription factor Tbr-1 [110]. How histone chaperones select the gene locus in the genome at which histones should be deposited will need to be resolved by studies of the binding specificity between histone chaperones and DNA binding factors. Elucidation of the fundamental molecular mechanisms underlying the action of NAP1 will help to resolve these issues.

TAF-Iβ/SET

TAF-Iβ (template activating factor Iβ) is one of the most well-characterized histone chaperones in the NAP1 family. TAF-Iβ has also been referred to as

SET, PHAPII, INHAT, IGAAD, StF-IT-1, and I2PP2A, depending on how it was isolated. The TAF-Iβ gene was first identified as a fusion of the *set* (SE translocation) gene to the *can* gene in acute undifferentiated leukemia [127]. TAF-I was also independently identified from a HeLa S3 cell extract as a stimulator of DNA replication from the Ad core, which is a chromatin-like structure composed of adenovirus DNA and viral basic proteins [128]. Subsequently, nucleotide sequence analysis revealed that TAF-I was identical to SET [129]. TAF-I exists in two isoforms, TAF-Iα and TAF-Iβ, which are generated by alternative splicing. The two isoforms are identical except for a short N-terminal sequence (1–37 aa of TAF-Iα and 1–24 aa of TAF-Iβ). TAF-Iβ is highly conserved in frog, mouse, rat, and human. TAF-Iβ homologues are also found in invertebrates. Spr-2, which has approximately 38% sequence identity to human TAF-Iβ, has been identified in *C. elegans* [130]. *Saccharomyces cerevisiae* Vps75, which was very recently identified as a NAP1 family protein, is similar to TAF-I in its domain structure (Fig. 1a) and in its preference for binding histones H3-H4 [131]. Vps75 interacts with novel histone acetyltransferase Rtt109 and activates its enzymatic activity [132]. Because TAF-I has significant amino acid sequence similarity to NAP1, its nucleosome assembly activity was tested and confirmed. TAF-I can be replaced by NAP1 in activation of the cell-free Ad core transcription system [107]. TAF-Iβ also exhibits sperm chromatin decondensation activity, similar to NAP1 [133]. Thus, TAF-Iβ is functionally related to NAP1.

The crystal structure of TAF-IβΔC (1–225 aa), which is composed of the N-terminal helix, the backbone helix, and the earmuff domain, forms a dimer that assumes a headphone-like structure similar to that of NAP1 (Fig. 1b) [106, 134]. However, the two histone chaperones differ structurally in several respects. First, the disposition of the N-terminal α1 helix is different in the two proteins. Helix α1 of NAP1 interacts with the main body of the dimer, but helix α1 of TAF-Iβ does not. Secondly, the α7 helix of TAF-Iβ is about 10 residues shorter than that of the corresponding helix of NAP1. Thirdly, NAP1 has an additional helix that is inserted between the backbone helix and the earmuff domain. Fourth, the relative orientations of the backbone helix and the earmuff domain differ. An outward rotation of ~40° is required to superimpose the earmuff domain of TAF-Iβ onto the corresponding domain of NAP1. These structural differences between TAF-Iβ and NAP1 may have functional consequences for histone binding specificity.

The structure-function relationships of TAF-Iβ have been intensively studied on the basis of the crystal structure. Interaction between TAF-Iβ and histones has been confirmed *in vivo* [135] and *in vitro* [136, 137]. TAF-Iβ preferentially binds to histones H3 and H4, although all core histones can bind to TAF-Iβ. Full-length TAF-Iβ binds to all four core histones, whereas the TAF-IβΔC deletion protein, which lacks the C-terminal acidic stretch, only binds histones H3 and H4 *in vitro* [134]. Since TAF-IβΔC has the same level of nucleosome assembly activity as the full-length protein [134], the activity seems to be achieved primarily by the interaction with histones H3 and H4. A biochemical study with TAF-Iβ mutants showed that the lower part of the earmuff domain is used for binding both core histones and dsDNA [134]. In addition, mutants with impaired histone and DNA binding activities have weak histone chaperone activity [134]. An interaction assay using histone tail peptides or acetylated histones purified from HeLa cells has shown that TAF-Iβ specifically binds to unacetylated, hypoacetylated, and repressively marked histones but not to hyperacetylated histones [138, 139]. TAF-Iβ has also been isolated as a subunit of the INHAT complex, a multiprotein complex that potently inhibits the histone acetyltransferase activities of p300/CBP and PCAF [135]. These observations suggest that the charged histone tail region is a major determinant in allowing TAF-Iβ (INHAT) to bind to chromatin and to play a regulatory role in chromatin modification.

TAF-Iβ has also been isolated by affinity purification of factors interacting with the DNA-binding domain of the transcription factors KLF5 and Sp1 [140, 141]. TAF-Iβ inhibits the DNA binding activities of both KLF5 and Sp1 and interferes with their transactivation activities [140, 141]. In addition, TAF-Iβ interacts with nuclear receptor-type transcription factors such as estrogen receptor α (ERα), progesterone receptor B, thyroid receptor β, and RXR α [142]. Other examples of the involvement of TAF-Iβ in promoter-specific transcriptional regulation have been reported. TAF-Iβ regulates transcription along with other transcription factors such as COUP-TF (chicken ovalbumin upstream promoter transcription factor), NGF-IB (nerve growth factor inducible protein B), and SF-1 (steroidogenic factor-1) to regulate P450c17 gene-specific transcription [143]. TAF-Iβ regulates the KAI1 gene-promoter-specific transcription by binding to the adaptor protein Fe65 [144]. Thus, TAF-Iβ may be recruited to specific genes through its interaction with specific DNA binding factors and may contribute to transcriptional regulation through its nucleosome assembly activity.

TAF-Iβ also has roles in phenomena such as gene translocation [127, 145]; caspase-independent apoptosis, which depends on its digestion by the trypsin-

like protease GzmA [146–149]; cell-cycle regulation, which depends on its interaction with the CDK inhibitor [150, 151]; and cancer regulation, which depends on binding to leukemia-related factor [152]. We propose that the basis of the multifunctional properties of TAF-Iβ is its histone chaperone activity, which plays a role in nucleosome assembly and disassembly. In the future, understanding the significance of the structural and functional separation between TAF-Iβ and NAP1 family proteins in individual cells will broaden our understanding of the roles of histone chaperones in multi-cellular organisms.

CAF-1

In 1986, Stillman and colleagues [153] identified an activity that preferentially assembles nucleosomes onto replicating DNA. In 1989, these researchers purified a factor possessing this activity and named it "chromatin assembly factor 1" (CAF-1) [154]. In 1995, they cloned cDNAs encoding the CAF-1 subunits p150 and p60 and showed that these subunits were sufficient for CAF-1 activity [155]. Subsequently, CAF-1 homologues were identified in both *Drosophila* [156–158] and yeast [159, 160], suggesting that CAF-1 promotes DNA replication-dependent nucleosome assembly in an evolutionarily conserved manner.

Human CAF-1 (hCAF-1) is composed of three conserved subunits: p150, p60, and p48. hCAF-1 binds histones H3 and H4 preferentially [155]. The complex of CAF-1 and histones H3-H4 has been designated the CAC complex [161]. Human p150, *Drosophila* p180, and yeast Cac1/Rlf2 share primary structure homology [155, 158–160]. The C-terminal region of p150 is involved in a dimer formation and interacts with p60 [155, 162]. These interactions are essential for CAF-1 activity (Fig. 1a). Moreover, the N-terminal region of p150 directly interacts with the DNA polymerase clamp PCNA [163, 164], and p150 colocalizes at DNA replication foci with PCNA and p60 in the S phase [163, 165, 166]. In the late S phase, p150 also interacts with the heterochromatin protein HP1 and PCNA, and localizes to heterochromatin [167]. Since p150 plays a key role in the interaction between CAF-1 and histones [155], it is likely to be a key component of CAF-1 in DNA replication-dependent nucleosome assembly.

Human p60, which is encoded in the Down's syndrome region on human chromosome 21 [168], possesses a HIRA-like amino acid sequence [169, 170]. p60, *Drosophila* p105, and the yeast homologue Cac2 contain a WD40 domain and a B-domain-like motif (Fig. 1a) [155, 158, 159, 169, 171] and binds to CIA directly, similar to HIRA [158, 172–174]. p60 contains several consensus target sites for various kinases [155, 175] and is phosphorylated by Cyclin/Cdk *in vitro* [176]. Phosphorylation of p60 [177] correlates with the cell-cycle-dependent regulation of CAF-1 activity and localization [166]. Active CAF-1 is present in the nucleus during the G1, S, and G2 phases, whereas inactive CAF-1 is present in both nucleus and cytoplasm during the M phase [166]. p60 is phosphorylated and associates with chromatin in response to UV irradiation, and CAF-1 promotes nucleosome assembly on newly synthesized DNA after UV irradiation [175, 178]. These observations suggest that p60 is a cell-cycle- and UV-irradiation-dependent regulatory subunit of CAF-1.

Human p48, *Drosophila* p55, and yeast Cac3/Msi1 contain a WD40 domain like p60 (Fig. 1a) [157, 159, 161, 179]. In addition, p48 is present not only in the CAF-1 complex but also in several chromatin-related complexes including the HAT1 complex [175], the HDAC1 complex [157], the histone methyltransferase complex ESC-E(Z) [180], and the ATP-dependent nucleosome remodeling complex NURF [181]. Only a small fraction of p48 is associated with the CAF-1 complex; the vast majority is present in other complexes [161, 166]. Moreover, p48 is associated with histone H4 in the absence of other CAF-1 subunits [161, 175], suggesting that it links predeposited histones and various chromatin-related complexes.

CAF-1 is involved in DNA replication/repair-dependent nucleosome assembly [182]. The proposed mechanism is as follows. First, CAF-1 is recruited onto replicated DNA, or DNA containing single-strand breaks, through PCNA in an ATP-dependent manner [163, 164]. The recruitment of CAF-1 to DNA damage sites is mediated by Werner syndrome protein WRN, which belongs to the RecQ family of DNA helicase [183]. Second, the CAF-1-mediated nucleosome assembly reaction proceeds in two steps. CAF-1 first deposits histones H3 and H4 onto replicated DNA, and histone H2A-H2B dimers subsequently bind to the histone $(H3\text{-}H4)_2$ tetramer [177]. In the first step, CAF-1 selectively binds to histone H3.1 [19] and then facilitates DNA replication/repair-dependent nucleosome assembly reaction coordinately with CIA *in vitro* [19, 173, 184, 185]. Third, CAF-1 forms a complex with methyl CpG binding protein MBD1 and H3-K9 methyltransferase SETDB1 in the S phase [186]. Fourth, p150 colocalizes with PCNA and HP1, which recognizes methylated H3-K9, to replicated heterochromatin regions [167]. It is notable that mutations in Cac1 and Pol30, the yeast homologues of p150 and PCNA, respectively [159, 160, 187], cause defects in telomere silencing. Although a functional effect on heterochromatin at telomeres is observed in the *cac1* mutant, there is no structural effect on

telomeric heterochromatin [188], suggesting that CAF-1 possesses an unknown function that regulates heterochromatin-specific gene expression.

CAF-1 cooperates with factors such as histone H3.1, CIA, PCNA, MBD1, SETDB1, and HP1, to facilitate nucleosome assembly on daughter DNA during replication of both euchromatin and heterochromatin. In addition, CAF-1 localizes near replication forks and transfers epigenetic information encoded on parental nucleosomes to daughter nucleosomes. CAF-1 is associated with histone H4 acetylated at some N-terminal tail residues, namely lysines 5, 8, or 12, acetylation patterns that are characteristic of newly synthesized histones. The N-terminal tail regions of histones H3 and H4, however, are not required for interaction with CAF-1 or for CAF-1-mediated nucleosome assembly [189]. Since these acetylations, which appear to function as markers of newly synthesized histones, can be recognized by other chromatin factors, the discrimination between newly synthesized and old histone proteins might be utilized in some nuclear events. The acetylation of the newly synthesized histones appears to be removed in daughter chromatin, and the parental histone modifications are then recovered by an unknown mechanism. Studies of the functional roles of CAF-1 in nucleosome replication will be an important issue for elucidating the mechanisms underlying the inheritance of epigenetic information.

HIRA

HIRA was isolated as a protein encoded by a gene within the DiGeorge syndrome critical region of human chromosome 22q11 in 1993 [190]. HIRA was originally named TUPLE1 because of a sequence similarity to the yeast corepressor Tup1 and *Drosophila* E(sp1) [190]. Osley, Lipinski, and colleagues [191–193] showed that TUPLE1 was more similar to Hir1 and Hir2/Spt1, which are repressors of histone gene transcription in yeast, and they renamed the protein HIRA (histone regulatory homolog A) [194]. Furthermore, Almouzni and colleagues [195] showed that HIRA has histone chaperone activity, on the basis of its histone binding activity [196–198].

HIRA's primary structure is divided into an N-terminal Hir1-like region and a C-terminal Hir2-like region (Fig. 1a) [170, 194]. Both HIRA and Hir1 possess two similar domains, a WD40 domain and a B-domain [170, 193, 194], which respectively show sequence similarity to those found in the CAF-1 p60 subunit [169, 171]. HIRA and Hir2 share a similar domain named the C-domain [170, 194]. A recent tertiary structural analysis of a B-domain peptide in complex with CIA has shown that the B-domain peptide forms an antiparallel β-hairpin (Fig. 1b) [171]. In yeast, Hir1 interacts with Hir2 through the C-terminal region of Hir1 [199, 200]. The two proteins associate with Hir3/Hpc1 and Hpc2 [201], forming the HIR complex, which assembles nucleosomes *in vitro* similar to HIRA in higher eukaryotes [202, 203]. These results suggest that HIRA may be a fusion of Hir1 and Hir2.

The HIR complex is functionally related to the SWI/SNF complex. Hir1 and Hir2 repress [191, 199] the promoter activity of the histone genes that are specifically expressed in the S phase [204–207]. The ATP-dependent nucleosome remodeling complex SWI/SNF is required for the expression of the histone genes and is recruited to this locus through its interaction with Hir1 and Hir2 [208]. The HIR complex, however, inhibits the nucleosome remodeling activity of the SWI/SNF complex *in vitro* [202], suggesting that histone gene expression is negatively regulated by the HIR complex and positively regulated by the SWI/SNF complex in a cell-cycle-dependent manner. HIRA is phosphorylated by Cyclin/Cdk in the S phase [209] and, in mitosis, HIRA dissociates from chromatin through mitotic phosphorylation [210].

Interactions between HIRA and transcription factors like Pax3 and Pax7 [198]; functional interactions with transcription elongation factors such as Spt4, 5, 6 and 16 [200, 211]; and the Spt^- phenotype of *hir1* and *hir2* mutants [192] suggest that HIRA, Hir1 and Hir2 are involved in transcription of several classes of genes. Since HIRA promotes replication-independent nucleosome assembly, it may be involved in chromatin assembly on promoters and ORFs. HIRA also associates with silencing factors. It interacts with CAF-1 p48 and with HDAC2 through the N-terminal WD40 domain and the C-terminal LXXLL motif, respectively [212]. Yeast phenotypic analyses showed that both Hir1 and Hir2 are involved in Cac1- and CIA-mediated gene silencing, although the deletion of either *HIR1* or *HIR2* alone has no effect on silencing [169, 185, 213]. HIRA is also known to be required for the recruitment of HP1 to pericentromeres in human cells [214] and to be involved in silencing at the mating loci and centromeres in *Schizosacccharomyces pombe* [215].

Human HIRA, yeast Hir1, and yeast Hir2 interact with human and yeast CIA, respectively [171, 185, 213, 216, 217]. Human HIRA and yeast HIR complex coordinately facilitate replication-independent nucleosome assembly reaction with CIA *in vitro* [19, 203]. In addition, both Hir1 and CIA are involved in the reassembly of nucleosomes on the yeast *PHO5* promoter after gene expression [218]. HIRA selectively binds to histone H3.3 [19] and is required for the deposition of histone H3.3 during sperm nucleus

decondensation in fertilized *Drosophila* eggs [219] and mouse zygote [220]. These results all indicate that HIRA and CIA are coordinately required for replication-independent nucleosome assembly *in vivo*. Structural and functional analyses of the interaction between HIRA and CIA [171, 216, 217] have identified their interaction surfaces, which are distinct from the sites of interaction between CIA and the histone H3-H4 dimer (Fig. 1b) [221–224], suggesting that HIRA and CIA may simultaneously associate with histone H3.3. Further structural and functional analyses of HIRA with the CIA-H3.3-H4 complex will clarify the mechanisms of nucleosome assembly and disassembly.

CIA/Asf1

Sternglanz and colleagues [225] isolated anti-silencing function 1 (Asf1) in a yeast genetic screen as a factor whose overexpression disrupts both telomeric and mating-type silencing. Kadonaga and colleagues [184] identified the replication-coupling assembly factor (RCAF), a complex composed of Asf1 and histones H3 and H4, as a CAF-1 stimulator from *Drosophila* embryo extracts. We isolated CCG1-interacting factor A (CIA), a human Asf1 homologue, as a factor that interacts with the bromodomain (BrD) of the largest subunit CCG1, a component of the general transcription factor TFIID, and biochemically identified it as a histone chaperone [221, 226].

CIA is composed of two structurally distinct domains, an evolutionarily conserved N-terminal core domain and a divergent C-terminal domain (Fig. 1a) [184, 221]. The N-terminal core domain possesses an immunoglobulin fold structure (Fig. 1b) [216, 222, 227] and is sufficient for histone chaperone activity [216, 228]. The C-terminal species-specific region of the yeast homologue is rich in acidic amino acid residues [225, 228]. However, in the human protein, the C-terminal region is enriched in serine and threonine residues [173, 221, 229] that include some phosphorylated sites [174]. Although there is only one gene in yeast and *Drosophila*, there are two CIA genes in vertebrates, plants, and *C. elegans*. The vertebrate proteins are CIA-I/Asf1a and CIA-II/Asf1b [173, 229, 230], which functionally differ in several respects: (i) HIRA preferentially interacts with CIA-I [19, 171, 217], (ii) CIA-I is more strongly phosphorylated than CIA-II in the C-terminal serine/threonine-abundant region [229], and (iii) CIA-I is ubiquitously expressed, but CIA-II is expressed in a tissue-specific manner [230].

During transcription in yeast and human, CIA interacts with the CCG1 BrD [226] and the histone H3.3 chaperone HIRA (Fig. 1b) [171, 185, 213, 216, 217]. In addition, another experiment suggests that CIA is likely to be a direct regulator of global gene expression [231]. CIA also targets the promoters and ORFs of inducible genes and participates in nucleosome disassembly and reassembly *in vivo* [218, 232–234]. In DNA replication, CIA associates with the histone H3.1 chaperone CAF-1 through its p60 subunit in an evolutionarily conserved manner [158, 172–174]. In addition, the ATPase complex RFC (replication factor C), which loads the DNA polymerase clamp factor PCNA, directly interacts with CIA and recruits CIA to the DNA *in vitro* [235]. Since CIA localizes at active replication forks, RFC is thought to recruit CIA to these sites *in vivo* [236]. During DNA repair, CIA interacts physically and functionally with the FHA domain of Rad53, which is a conserved checkpoint protein kinase [237–239]. This interaction inhibits the histone chaperone activity of CIA. Although it was reported that phosphorylation of Rad53 is involved in its interaction with CIA, this notion was later dismissed [237, 239]. These results all show that CIA is involved in various DNA-specific reactions including transcription, DNA replication, and DNA repair.

Phenotypic analyses of yeast also support the above conclusion. The CIA disruptant shows an Spt^- phenotype [185, 226] and is sensitive to HU, MMS, BLM, camptothecin, and cisplatin [184, 225, 240]. In addition, it exhibits an increased frequency of homologous recombination [241]. Furthermore, the mutation of genes encoding chromatin factors such as CAF-1 subunits, PCNA, and Sir1 confers defects in telomeric and mating-type silencing in CIA disruptants [184, 185, 213, 225, 242, 243]. These phenotypic studies suggest that CIA is also involved in DNA recombination and silencing. CIA is regulated in a cell-cycle-dependent manner as follows. CIA is expressed in the S phase in yeast [225]. CIA has cell-cycle-specific chromosomal and cytoplasmic localization in *Drosophila* [244], and is phosphorylated by the S-phase-specific kinase Tlk in human, *Arabidopsis*, and *Drosophila* cells [229, 245, 246]. Disruption of CIA results in a growth defect in *S. cerevisiae* and lethality in *S. pombe* [184, 225, 228]. Dead cells of the *S. cerevisiae* CIA disruptant show an apoptosis-like phenotype [247]. In human fibroblast cells, the formation of senescence-associated heterochromatin foci (SAHF) is driven by CIA-I and HIRA [217, 248]. These observations suggest that CIA is involved in a variety of cell functions including cell proliferation, death, and senescence. In addition, CIA might be related to cell differentiation, because the expression patterns of CIA-I and CIA-II are different in various human cells [230].

CIA interacts with the C-terminal region of histone H3 [221–224]. This interaction plays a central role in

transcription, DNA replication, and DNA repair [223, 224]. In the nucleosome assembly reaction, CAF-1 and HIRA cooperate with CIA in replication/repair-dependent and replication-independent manners, respectively [19, 173, 184, 185, 203]. CIA forms a stable complex with histones H3 and H4 *in vivo* and *in vitro* [184, 221, 249]. Interestingly, this complex is composed of one CIA protein and one histone H3-H4 dimer [249]. This result suggests two possibilities: either that the CIA-H3-H4 complex is formed before deposition of the histone H3-H4 complex onto DNA, or that the complex is produced by disruption of the histone $(H3\text{-}H4)_2$ tetramer. Surprisingly, the histone $(H3\text{-}H4)_2$ tetramer, which for the past 30 years was believed to be robust and stable, is dissociated into two histone H3-H4 dimers by CIA (Fig. 2a) [224]. Structural analyses of the CIA-H3-H4 complex [223, 224] and CIA-H3 peptide complex [250, 251] indicate that the interaction of the histone H3-H4 dimer with another histone H3-H4 dimer and that with CIA are mutually exclusive options. In HeLa cells, the formation of this complex causes CIA to pool histones in the cytoplasm [252]. The complex seems to constitute the core of a predeposited-histone complex composed of CIA and additional histone chaperones in the nucleus [19]. These results suggest that CIA-H3-H4 complexes formed in the cytoplasm are imported into the nucleus and deposit histones onto DNA together with other histone chaperones [223, 224]. After histone delivery, free CIA may cause nucleosome disassembly through its histone $(H3\text{-}H4)_2$ tetramer-disrupting activity [224].

CIA interacts with histone modification enzymes and modification-recognizing proteins/domains. CIA interacts with the SAS complex that acetylates the histone N-terminal tail region [242, 243], and represses the HAT activity of this complex [253]. In contrast, CIA directly activates the HAT activity of Rtt109, which acetylates histone H3-K56 in the C-terminal core domain [254–256], through interaction with Rtt109 [132]. Since histone H3-K56 is exposed in the CIA-H3-H4 complex [223, 224], CIA is able to recruit Rtt109 to H3-K56 efficiently. Deletion mutants of CIA and Rtt109 increase supercoiled plasmid DNA, compared to the wild type [254]. A mutation mimicking acetylated histone H3-K56 reduces the initial FRET signal between two linker DNAs on the nucleosome [257]. These results suggest that acetylation of histone H3-K56 is involved in structural changes in the nucleosome. Furthermore, CIA interacts with the CCG1 BrD [226], which recognizes a histone-acetylated lysine residue, implying that CIA is involved in regulating gene expression and translating histone modification patterns into chromatin structure alterations (Fig. 2b).

Structural and functional analyses of the CIA-histone H3-H4 complex show that CIA probably plays a general role in nucleosome assembly and disassembly during a variety of DNA-mediated reactions. On the other hand, CIA is involved in nucleosome assembly in yeast [203, 241], *Drosophila* embryo [184], and HeLa cell extract [173, 252] but not in *Xenopus* egg extract [258]. These diverse functional roles might be affected by the differences in CIA-associated molecules from different sources. Since CIA localizes to the replication fork and dissociates the histone $(H3\text{-}H4)_2$ tetramer into two histone H3-H4 dimers, it may participate in the inheritance of epigenetic information encoded as histone modifications, and the regulation of CIA activity may be critical for cell fate choices such as proliferation or differentiation (Fig. 2d). Demonstration of these hypotheses awaits experimental confirmation.

FKBP

Peptidyl-prolyl cis/trans isomerase (PPIase) is an enzyme that catalyzes proline isomerization and alters the orientation of peptide chains at proline residues. The FK506-binding protein (FKBP), a member of the PPIase family, was named for its ability to bind the immuno-suppressive drug FK506. Most FKBPs have additional domains that interact with other factors and/or have other functions. Notably, nuclear FKBP, which contains two acidic regions and one basic region in the N-terminal domain, has nucleosome assembly activity (Fig. 1a) [259].

The first nuclear FKBP, the FK506-binding proline rotamase 3 (Fpr3), was independently isolated in *S. cerevisiae* by three groups in 1994 as an abundant nucleolar protein that recognizes the NLSs of histone H2B [260–262]. Fpr4 was identified as an Fpr3 family member in *S. cerevisiae* [263]. Analysis of the *S. pombe* Fpr3 homologue SpFkbp39p suggested that nuclear FKBPs interact with DNA and histones through their N-terminal highly basic and acidic regions, respectively [264]. Subsequently, the nucleosome assembly activity of nuclear FKBP was identified [259]. Although the FKBP PPIase domain is conserved from *Escherichia coli* to human, full-length nuclear FKBP is conserved only from yeast to insects [264] and is not found in vertebrates. However, a BLAST search showed that the N-terminal domain of Fpr3 shares a great degree of homology (36 % amino acid sequence identity) with nucleolin, a mammalian protein localized in the nucleolus (Fig. 1a) [261]. Nucleolin also has histone transfer activity [265], suggesting that nucleolin and nuclear FKBPs have similar functional roles.

The nucleolar localization of FKBPs is characteristic of these histone chaperones [259–261, 264]. Although

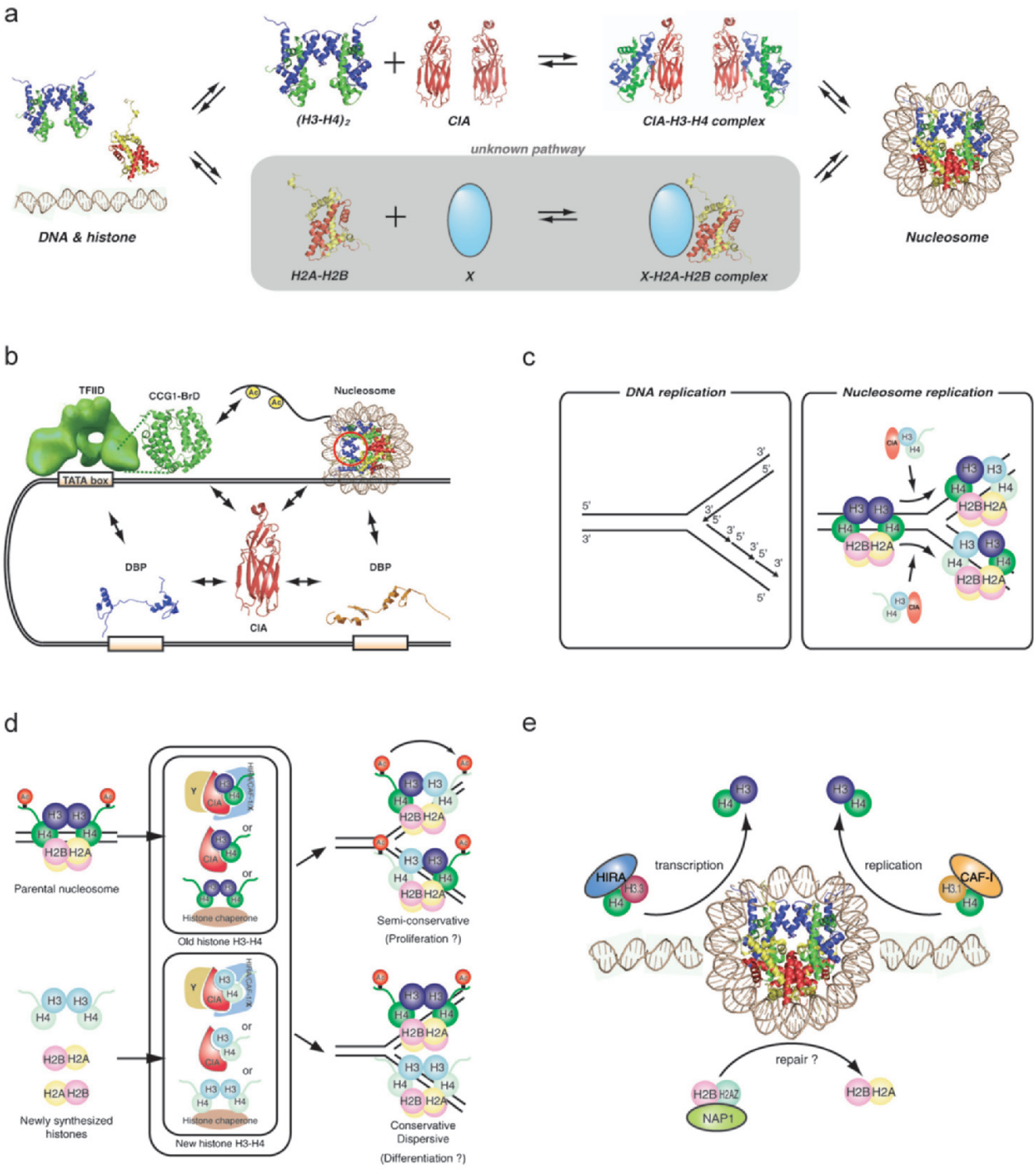

Figure 2. Future directions for histone chaperone research. (*a*) Intermediates in nucleosome assembly/disassembly reactions. (*b*) Interactions of CIA with the acetylated histone recognition domain BrD. DNA binding factors and nucleosomes will reveal pathways from signal recognition to alteration of nucleosome structure. (*c*) Comparison of conventional DNA replication mechanism with the hypothetical CIA-mediated nucleosome replication mechanism. (*d*) Hypothetical model of nucleosome formation during DNA replication. Regulation of the CIA-H3-H4 complex formation by dissociation of the histone $(H3\text{-}H4)_2$ tetramer into two H3-H4 dimers by CIA supports the hypothesis that epigenetic information is maintained or converted through both histone modifications and the state of histone variants. (*e*) Exchange reactions for histone variants in nucleosomes. These model reactions are supported by histone variant-specific histone chaperones.

many nucleolar proteins in *S. cerevisiae* are complexed with Net1, which maintains their localization, the nucleolar localization of Fpr3 does not depend on Net1 [266]. Fpr3 and the ribosomal protein S24 were isolated by FK520 (an FK506 derivative) affinity column chromatography [262], and a yeast two-hybrid assay showed that both Fpr3 and Fpr4 interact with S24 [263]. These observations suggest that Fpr3 and Fpr4 contribute to nucleolar function.

The PPIase activity of nuclear FKBPs may be involved in histone modification and histone variant exchange. Fpr4 interacts with core histones through its N-terminal histone chaperone domain and preferentially binds the histone H3-H4 complex rather than histone H2A-H2B [267]. Fpr4 specifically binds the N-terminal tail K/RxxGGK motif of both histones H3 and H4, leading to isomerization of the histone H3-P38 residue, which is located close to the binding motif. This conformational change in histone H3 regulates the methylation of histone H3-K36 by the methyltransferase Set2 [268]. In the *S. cerevisiae nap1Δ chz1Δ* strain, both Fpr3 and Fpr4 are found in the histone variant H2AZ complex [269], suggesting that Fpr3 and Fpr4 are involved in the histone H2AZ exchange reaction.

Further functional analyses have implicated Fpr3 in cell-cycle regulation. Reversible phosphorylation of Fpr3 plays a role in regulating the growth rate and budding of *S. cerevisiae* [270–272]. Fpr3 interacts with PP1 (yeast PP2A: protein phosphatase 2A) through its PPIase domain, and this interaction regulates PP1 localization and maintains recombination checkpoint activity *in vivo* [273]. Fpr3 suppresses Tom1 (temperature-dependent organization in mitotic nucleus) phenotypes, which include defects in nuclear division, the maintenance of nuclear structure, and nucleocytoplasmic transport at high temperatures [274–276]. Therefore, Fpr3 appears to play a role in cell-cycle regulation.

Nuclear FKBPs are unique histone chaperones whose PPIase catalytic activity induces the structural conversion of target proteins. The peptidyl-prolyl isomerase domain, which is conserved among all FKBPs, inhibits nucleosome assembly [259, 267] but its role in this process is not understood. Previous findings indicate that the mechanisms of the elementary reactions in nucleosome assembly should be analyzed to acquire an understanding of the relationship between PPIase activity and nucleosome assembly activity of nuclear FKBPs. This analysis would broaden our understanding of the functional roles played by nuclear FKBPs, including chromatin remodeling in the nucleolus, ribosomal synthesis, and cell-cycle regulation.

JDP2

JDP2 (Jun dimerization protein 2) was isolated as a member of the AP-1 protein family that interacts with c-Jun and ATF-2 [277, 278]. AP-1 family proteins are DNA binding factors that typically form homodimers or heterodimers with other members of the AP-1 family. Surprisingly, JDP2 was found to bind histones and to have histone chaperone activity [279].

JDP2 has a bZIP region composed of a basic region involved in DNA binding and a leucine zipper for dimer formation (Fig. 1a) [277]. The C-terminal region contains a site phosphorylated by JNK and p38, which are kinases that phosphorylate a number of AP-1 family members [280, 281]. The N-terminal region of JDP2, however, does not contain the transcriptional activation domains that are generally shared among AP-1 family members [282]. JDP2 forms homodimers and/or heterodimers with AP-1 family members such as c-Jun, JunB, JunD, ATF-2, and C/EBPγ [277, 278, 283]. The JDP2-c-Jun and JDP2-ATF-2 complexes have been analyzed in detail. These complexes bind DNA and cause transcriptional repression [277, 278]. The JDP2-ATF-2 complex specifically interacts with the histone deacetylase HDAC3 on a specific DNA element in the retinoic acid-induced *c-jun* promoter. This complex replaces a transcriptional activation complex composed of ATF-2 and the histone acetyltransferase p300, and appears to repress transcription [284].

In addition, JDP2 itself inhibits the HAT activity of p300. The INHAT activity of JDP2 requires both the histone binding and the DNA binding basic regions [279]. Since this inhibition does not require DNA, the DNA binding basic region of JDP2 may inhibit HAT activity in a manner that does not involve DNA binding. JDP2 also promotes transcriptional activation. JDP2 interacts with the DNA binding progesterone receptor (PR) through their respective DNA binding domains, and this complex activates PR-induced gene expression by localizing on promoters [282]. JDP2 stabilizes the N-terminal activation domain of PR [285], which is not by itself stable but which is required for activation [282]. JDP2 is also involved in cell differentiation [284, 286, 287], tumorigenesis [288, 289], and cell death [290, 291], similar to other AP-1 family members. These biological processes may be mediated by JDP2 through an alteration of chromatin structure.

JDP2 is a unique histone chaperone that also has DNA binding activity. Further analysis may reveal that other DNA binding factors have histone chaperone activities as well. In addition, some histone chaperones and histone-associated factors may, in the future, be shown to have DNA binding activities. Isolation and characterization of these factors will

provide new insights into histone-associated factors and DNA binding factors.

Chaperone-like factors

Thus far, we have discussed ten types of histone chaperones whose functions are well characterized. In this section, we introduce other histone chaperones or chaperone-like factors whose functions are relatively uncharacterized.

Drosophila DF31 was isolated as a factor that decondenses *Xenopus laevis* sperm chromatin [292]. DF31 is a small protein that is enriched in charged amino acids [293], and it binds to each of the four core histones with equal affinity [292]. Injection of antisense oligonucleotides against DF31 mRNA into embryos severely disrupts the nuclear structure [293].

The yeast Rtt106 gene was genetically isolated as a suppressor mutant against the silencing defects conferred by mutation of Pol30 (the yeast homologue of PCNA) or Cac1 (the yeast homologue of CAF-1 p150) [294]. Rtt106 interacts with histones H3-H4 and has a primary structure similar to that of human SSRP1, which is a subunit of the FACT (facilitates chromatin transcription) chromatin transcription elongation complex (see below). Rtt106 also interacts with Cac1 and Sir4. *rtt106 cac1* double mutant shows heterochromatin silencing defect, suggesting that Rtt106 cooperates with CAF-1 in replication-dependent nucleosome assembly [294, 295].

There are other histone chaperone-like factors that do not strictly adhere to the two criteria we used to define histone chaperones. FACT [296] and nucleolin [265] represent histone binding proteins as defined by criterion (i). However, the histone deposition assay that has been used to judge the nucleosome assembly activities of these two proteins differs from the method that is proposed in the present manuscript [criterion (ii)]. In that assay, DNA is incubated with core histones in the presence or absence of the candidate histone chaperone, and differences in the speed of DNA migration are measured in an electrophoretic mobility shift assay. This assay shows their histone deposition activities onto DNA. These results cannot answer whether or not they assemble nucleosomes.

The FACT subunit Spt16/Cdc68 was initially identified as a transcription- and cell division cycle-related factor in a yeast genetic screen [297–300]. Two DNA polymerase α-interacting factors, Spt16/Cdc68 and a novel protein Pob3, were isolated by affinity chromatography [301]. Pob3 bound to Spt16/Cdc68 and possessed sequence similarity to a mouse HMG-like factor [301]. These results suggested that Spt16 and Pob3 are involved in the regulation of chromatin structure. The FACT complex, which is composed of the HMG-box-containing protein SSRP1 and p140/hSpt16 [302], was isolated from HeLa nuclear extracts on the basis of its ability to facilitate RNA polymerase translocation along a nucleosomal template [303]. FACT was also isolated from *Xenopus* eggs as a DNA unwinding factor (DUF) that unwinds closed-circular duplex DNA in the presence of topoisomerase I, but does not possess a DNA gyrase activity [304]. Components of the FACT complex are highly conserved in all eukaryotes [305]. *S. cerevisiae* FACT (yFACT) is composed of three proteins: Spt16/Cdc68 (120 kDa), Pob3 (63 kDa), and Nhp6 (11 kDa). Pob3, the yeast homologue of SSRP1, lacks an HMG domain. This domain is presented to the complex by the small HMG-box proteins Nhp6a and Nhp6b [306, 307]. The association of the Spt16-Pob3 complex with chromatin depends on Nhp6 [307], and multiple Nhp6 molecules are required for yFACT recruitment to chromatin [308].

The pleiotropic functions of FACT are indicated by its involvement in transcription initiation, transcription elongation, DNA replication, and even DNA repair. For transcription initiation, FACT functions in establishing transcription initiation complexes. FACT interacts with the general transcription initiation factor TFIIE in an evolutionarily conserved manner [309]. yFACT stimulates TBP and TFIIA binding to a nucleosomal TATA site [310]. *Drosophila* FACT interacts with the GAGA factor to stimulate chromatin remodeling at the promotor [311] and to modulate chromatin structure to maintain gene expression [312]. FACT promotes transcription elongation by RNA polymerase II on chromatin templates *in vitro* [303] and associates with RNA polymerase II complexes throughout transcribed regions [93, 313, 314]. Histone H2B monoubiquitination functions cooperatively with FACT to regulate transcription elongation [315]. FACT binds directly to factors important for DNA replication, such as DNA polymerase α/primase [301] and the replicative helicase MCM [316]. Since FACT promotes the DNA unwinding activity of the MCM helicase on nucleosomal templates, the FACT-MCM interaction appears to be important for proper initiation of DNA replication [316]. For DNA repair, in response to UV-mediated DNA damage, FACT forms a complex with casein kinase II and phosphorylates S392 of p53 to activate DNA repair [317, 318]. This broad range of FACT functions may be needed to overcome the inhibitory effect of nucleosomes at many steps during chromatin-based processes. Several observations suggested that FACT may act as a histone chaperone-like factor to promote H2A-H2B dimer dissociation and allow RNA polymerase and DNA polymerase translocation through nucleosomes [302]. Nucleolin has been

suggested to work in a manner similar to FACT in the nucleolus [265].

Chz1 (chaperone for H2AZ-H2B 1) fulfills criterion (i) for a histone chaperone by its histone H2AZ-H2B binding activity [269], but its nucleosome assembly activity remains to be demonstrated. Chz1 may be implicated in the histone H2AZ exchange reaction as the factor that transfers the histone H2AZ-H2B dimer to the SWR1 complex [269].

Histone chaperone-like activity has been proposed for the nucleosome remodeling complexes ACF [319], RSF [320], and the ATPase MCM2 [321], based on histone binding and nucleosome assembly assays in reaction buffers containing ATP. ACF and RSF are thought to function in nucleosome assembly and nucleosome spacing in parallel. MCM2 is thought to be involved in replication-dependent nucleosome assembly. However, the role of ATP in the nucleosome assembly activities of these complexes remains to be elucidated.

It is of note that there is no consensus for defining histone chaperone activity. Due to this lack of agreement, new "histone chaperones", which have been classified as such using many different criteria, have been isolated. To gain a general agreement about the mechanistic action of histone chaperones, it will be necessary to dissect nucleosome assembly/disassembly reactions into elementary reaction steps and to identify the factors mediating each reaction step, as well as positive and/or negative regulators. This information would lead to a clearer definition of histone chaperones. We have begun to analyze the molecular mechanisms of nucleosome assembly/disassembly activities facilitated by histone chaperones. We now need to elucidate the structural and functional relationships between a variety of histone chaperones and other histone-associated enzymes/factors, and to determine the elementary reactions required for nucleosome assembly/disassembly to gain a broad but detailed perspective of the structural and functional roles of histone chaperones.

Mechanisms

The preceding sections summarized the structural and functional roles of histone chaperones. Here, we provide an overview of the mechanisms of nucleosome assembly and disassembly mediated by histone chaperones and forecast the directions of future research.

Analyses of nucleoplasmin, N1/N2, and CAF-1 show that the nucleosome assembly reaction proceeds in two distinct steps [40, 177]. One histone $(H3\text{-}H4)_2$ tetramer or two histone H3-H4 dimers are first deposited onto DNA, and two histone H2A-H2B dimers then bind to each side of the histone $(H3\text{-}H4)_2$ tetramer. Functional analyses of the histone H3.1 and histone H3.3 complexes [19] and structural analyses of the CIA-H3-H4 [223, 224] and CIA-HIRA complexes [171] show that the CIA-H3-H4 heterotrimer, together with other histone chaperones, comprises a key part of the predeposition complex. To form the histone $(H3\text{-}H4)_2$ tetramer from this complex, CIA must dissociate from the histone H3-H4 dimer. Factors such as CAF-1 and HIRA, which assemble nucleosomes synergistically with CIA [19, 173, 184, 185, 203], may bind to the CIA-H3-H4 trimer, remove CIA, and stimulate the formation of the histone $(H3\text{-}H4)_2$ tetramer complexed with the DNA. Moreover, other factors, such as FACT, NAP1, and nucleoplasmin, all of which preferentially bind to the histone H2A-H2B dimer, may supply two histone H2A-H2B dimers to each histone $(H3\text{-}H4)_2$ tetramer to form a new nucleosome.

The nucleosome disassembly reaction also proceeds in two steps, in the reverse of the nucleosome assembly reactions [322]. First, two histone H2A-H2B dimers are removed from nucleosomes by factors such as FACT, NAP1, and nucleoplasmin. CIA then disrupts the histone $(H3\text{-}H4)_2$ tetramer into two histone H3-H4 dimers. NAP1 induces H2A-H2B dimer dissociation from the nucleosome core particle (NCP). The dissociation of the histone H2A-H2B dimer seems to facilitate sub-nucleosome sliding [111]. This dissociation activity requires the C-terminal highly acidic region of NAP1, suggesting that this region transiently binds to histone H2A-H2B dimers rather than to nucleosomal DNA, and contributes to the dissociation of histone H2A-H2B dimers from the nucleosome. The C-terminal region of TAF-Iβ is required for binding the histone H2A-H2B dimer [134], suggesting that TAF-Iβ also displaces histone H2A-H2B dimers from the nucleosome in an NAP1-like manner. CIA binds to the C-terminal region of histone H4, which interacts with histone H2A in the nucleosome, and changes its conformation, causing disruption of the histone $(H3\text{-}H4)_2$ tetramer (Fig. 2a) [224]. The mechanism by which a large effect (tetramer disruption) originates from a small interaction (formation of a β-sheet) is similar to the essence of Japanese Judo (Yawara), "softness tames toughness", so we named this mechanism the "Yawara split" model in the spirit of Judo [224]. Other groups have analyzed the structure of the CIA-H3-H4 complex by coexpressing CIA/ASF1 and histones H3 and H4, and have hypothesized a mechanism for CIA-mediated nucleosome disassembly called the "strand capture split" model [223].

These lines of evidence clearly indicate that nucleosome assembly and disassembly reactions proceed through at least two major steps and that different histone chaperones function in each step. Therefore, it is important to determine in more detail which histone chaperones act at each step and how they function. Furthermore, it will be essential to isolate novel types of histone chaperones, to identify interaction surfaces between nucleosomes and histone chaperones, and to develop assays to analyze elementary reactions.

Do histone chaperones function only to promote nucleosome assembly and disassembly? Because histone chaperones bind to histones, we must consider the functional roles of histone chaperones throughout the histone life span, from synthesis to degradation. Large amounts of histone proteins are transported into the nucleus after protein synthesis. CIA is present in both the nucleus and cytoplasm, whereas CAF-1 and HIRA reside primarily in the nucleus [252], suggesting that CIA-H3-H4 complexes formed in the cytoplasm migrate into the nucleus and form the CIA-H3.1-H4-CAF-1 complex or the CIA-H3.3-H4-HIRA complex. Additionally, large amounts of nucleoplasmin are present in complexes with histones H2A and H2B in *Xenopus* eggs. Nucleoplasmin has an NLS, suggesting that it may transport the histone H2A-H2B dimer into the nucleus. NAP1 and TAF-Iβ also contain an NLS, so these proteins may also transport histones into the nucleus.

After nuclear entry, histones are deposited onto DNA, and nucleosomes and chromatin are formed with other histone- and/or DNA-associated factors. There are two types of deposition reactions. One is implicated in nucleosome replication, which occurs concomitantly with the progression of the DNA replication fork throughout the genome. The other is implicated in the exchange of histone variants during transcription and DNA repair in transcriptionally active loci and DNA-damaged loci, respectively. In nucleosome replication, examination of DNA replication-dependent nucleosome assembly [19, 184, 185] has indicated that CIA and CAF-1 coordinately deposit histones H3.1 and H4 onto replicated DNA. Subsequently, FACT may deliver the histone H2A-H2B dimer to the histone $(H3.1\text{-}H4)_2$ tetramer on a daughter DNA strand. In contrast, the exchange of histone H3.3 in transcriptionally active regions is probably mediated by CIA and HIRA, because they coordinately facilitate DNA replication-independent nucleosome assembly [19, 203]. In the case of the histone H2A variant H2AZ, the SWR1 chromatin remodeling complex catalyzes the replacement of conventional histone H2A with H2AZ in chromatin [20]. Because both NAP1 and Chz1 form a stable trimer with the histone H2AZ-H2B dimer as its major associating factor, they may transfer the histone H2AZ-H2B dimer to the SWR1 complex. In addition, the histone chaperones Fpr3 and Fpr4 also interact with H2AZ when Nap1 and Chz1 are absent, suggesting that they may also be involved in the exchange reaction [269].

Finally, the histone is terminated by degradation. Histones may function as radical scavengers and shield DNA from attack by reactive oxygen species [323]. Carbonylated histones, which have sustained damage, and surplus free histones remaining after exchange reactions involving histone variants, may be appropriately stored or degraded to prevent aggregation with histones and/or DNA. The histone degradation pathway remains relatively unknown, although polyubiquitinated histone H3 is known to be degraded by the 26S proteasome [324]. As some histone chaperones may interact with degradation factors, their functional roles in regulating histone degradation will become an important area of research because of the abundance and heterogeneity of histones.

As discussed thus far, there are several aspects of both the intracellular metabolism of histones and the elementary reactions of nucleosome assembly and disassembly processes. The need for many types of histone chaperones is underscored by the diversity of histone modifications and histone variants, the temporal-spatial regulation of the formation of chromosomal structural and functional region, and the stage/tissue-specific regulation of gene expression. There is now an urgent need to elucidate the unity and diversity among nucleosome assembly and disassembly reactions that occur during distinct DNA-mediated reactions, such as transcription, DNA replication, repair, and recombination, by understanding the diversity of mechanisms underlying the actions of the various histone chaperones.

Conclusions and perspectives

Independent studies characterizing the histone chaperones discussed here have provided the basis for our current understanding of histone chaperones. Each histone chaperone has a specific histone binding preference, and some histone chaperones preferentially bind to a histone variant. These preferences have provided insights into the mechanisms of nucleosome assembly and disassembly reactions, in which multiple histone chaperones act in a stepwise and synergistic manner. These observations suggest that each histone chaperone plays a unique role in these reactions.

The recent discovery of the histone $(H3\text{-}H4)_2$ tetramer-disrupting activity of CIA [224] and the elucidation

of the tertiary structure of the CIA-H3-H4 complex [223, 224] are breakthroughs that will influence the future direction of histone chaperone research. Based on the biochemical and structural results discussed in this review, we offer the following major predictions.
(i) Based on the finding that CIA disrupts the interaction between two histone H3-H4 dimers in a histone $(H3\text{-}H4)_2$ tetramer, we predict the isolation of the factor that disrupts the interaction between the histone $(H3\text{-}H4)_2$ tetramer and the histone H2A-H2B dimer in NCP. This factor should interact with the histone-$(H3\text{-}H4)_2$-tetramer's surface, which interacts with the histone H2A-H2B dimer. Analysis of the mechanism by which this factor disrupts $(H3\text{-}H4)_2$-H2A-H2B interactions would reveal further details of the mechanisms of nucleosome assembly and disassembly (Fig. 2a).
(ii) The functional link between signal transduction in the nucleus and the alteration of nucleosome structure will be clarified, as suggested by the interaction between CIA and the acetylated histone recognition domain, bromodomain (BrD) [226]. The structural and biochemical bases for the interactions between histone chaperones and histone-modification-recognizing domains will be determined. These interactions should provide insights into the relationship between the recognition of epigenetic modifications on histones and the alteration of nucleosome structure (Fig. 2b).
(iii) The mechanism underlying semi-conservative or conservative nucleosome replication [224] acting in concert with semi-conservative DNA replication [325, 326] will be revealed (Fig. 2c). Many histone chaperones have been shown to be factors that work cooperatively in DNA replication. We predict that nucleosomes on the parental DNA duplex will be disrupted and distributed to daughter DNA strands with the progression of the DNA replication fork. Histone chaperones that have nucleosome disrupting and assembling activities will work in this process.
(iv) The mechanism underlying the transfer of epigenetic modifications from parental to daughter cells will be elucidated. The mechanism that enables switching between semi-conservative and conservative nucleosome replication modes will be determined. The two replication modes may be switched by regulating the histone $(H3\text{-}H4)_2$ tetramer-disrupting activity of CIA at various genomic loci, depending on the physiological conditions of the cell (Fig. 2d).
(v) The mechanism underlying the exchange of histone variants during transcription and DNA repair will be identified. The mechanism that regulates the spatial and temporal patterns of histone variant exchange could be revealed by analyzing the interaction network between specific DNA binding factors and histone chaperones such as HIRA, CAF-1, and NAP1, which recognize specific histone variants (Fig. 2e).
(vi) Novel histone-related activities of histone chaperones will be revealed. Histone chaperones have not only nucleosome assembly/disassembly activities but also diverse histone-related activities such as histone shuttling, histone storage, histone variant exchange, histone modification regulation, linker histone deposition, nucleosome sliding, and so on. This functional diversity may have been acquired in the course of evolution because histone chaperones travel closely with histones. We predict that a number of histone-related activities of histone chaperones will be accumulated in the future.
It is important to analyze the activity and molecular mechanisms of histone chaperones by considering their functional significance as described above. The mechanism of action of the histone chaperone, in particular, should be analyzed in the context of a signaling network. Since nucleosome assembly and disassembly are among the most downstream effects of a signal transduction, the mechanism of signal processing and integration at the level of the histone chaperone will be a critical issue to address. Another critical issue will be the robustness of the signal processing/integration system in the cell. The functional and physical interaction network between histone chaperones and other chromatin factors [327], such as chemical modification enzymes targeting histones, ATP-dependent nucleosome remodeling factors, and sequence-specific DNA binding factors, will reveal the mechanisms of signal processing and gene regulation from the genetic and epigenetic viewpoints.
In this review, we have predicted the future research of histone chaperones based on the biochemical and structural studies undertaken thus far. It is notable that, as described above, the histone chaperones have been characterized by biochemical means, particularly through the supercoiling and MNase assays. What is the relationship between the activity of the supercoiling and MNase assays and *in vivo* physiological activities? Recent research suggests that the *in vitro* activity of the particular histone chaperone is not always observed under physiological conditions [258]. It is conceivable that the histone chaperone is not directly involved in nucleosome assembly/disassembly *in vivo*, but is involved in other specific function(s) with its histone binding activity. The elucidation and understanding of this possible discrepancy between *in vitro* and *in vivo* activities of histone chaperones is the next critical step in the study of histone chaperones. To elucidate the relationship between the two, a transdisciplinary approach is essential. First, three-dimen-

sional structural information on the histone chaperone would be valuable for addressing this issue. Second, the establishment of a new assay system for analysis of the elementary reaction steps is necessary to elucidate their molecular mechanisms. Third, the development of new methods and ideas for *in vivo* functional analysis using current genetics is awaited for the verification of *in vitro* histone chaperone activity. They must play roles in connecting the *in vitro* and *in vivo* activities of histone chaperones to compensate for their weak points. This will promote understanding of their respective roles in the nucleosome dynamics in cells.

Acknowledgements. We thank K. Hasegawa, Y. Hayashi, Y. Ogawa, M. Sakamoto, and N. Sano for helpful comments on the manuscript. Our work is supported by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan; the Mitsubishi Foundation; the Uehara Memorial Foundation; the Japan Science and Technology Agency (JST); and the New Energy and Industrial Technology Development Organization (NEDO) of Japan.

1 Kornberg, R. D. and Thomas, J. O. (1974) Chromatin structure; oligomers of the histones. Science 184, 865–868.
2 Axel, R., Melchior, W., Sollner-Webb, B. and Felsenfeld, G. (1974) Specific sites of interaction between histones and DNA in chromatin. Proc. Natl. Acad. Sci. USA 71, 4101–4105.
3 Oudet, P., Gross-Bellard, M. and Chambon, P. (1975) Electron microscopic and biochemical evidence that chromatin structure is a repeating unit. Cell 4, 281–300.
4 Laskey, R. A., Mills, A. D. and Morris, N. R. (1977) Assembly of SV40 chromatin in a cell-free system from *Xenopus* eggs. Cell 10, 237–243.
5 Laskey, R. A., Honda, B. M., Mills, A. D. and Finch, J. T. (1978) Nucleosomes are assembled by an acidic protein which binds histones and transfers them to DNA. Nature 275, 416–420.
6 Laskey, R. A. and Earnshaw, W. C. (1980) Nucleosome assembly. Nature 286, 763–767.
7 Bonne-Andrea, C., Harper, F., Sobczak, J. and De Recondo, A. M. (1984) Rat liver HMG1: A physiological nucleosome assembly factor. EMBO J. 3, 1193–1199.
8 Drew, H. R. (1993) Reconstitution of short-spaced chromatin from the histone octamer and either HMG-14,17 or histone H1. J. Mol. Biol. 230, 824–836.
9 Stein, A., Whitlock, J. P. Jr. and Bina, M. (1979) Acidic polypeptides can assemble both histones and chromatin *in vitro* at physiological ionic strength. Proc. Natl. Acad. Sci. USA 76, 5000–5004.
10 Díaz-Jullien, C., Pérez-Estévez, A., Covelo, G. and Freire, M. (1996) Prothymosin α binds histones *in vitro* and shows activity in nucleosome assembly assay. Biochim. Biophys. Acta 1296, 219–227.
11 Holler, E., Achhammer, G., Angerer, B., Gantz, B., Hambach, C., Reisner, H., Seidel, B., Weber, C., Windisch, C., Braud, C., Guerin, P. and Vert, M. (1992) Specific inhibition of *Physarum polycephalum* DNA-polymerase-α-primase by poly(L-malate) and related polyanions. Eur. J. Biochem. 206, 1–6.
12 Nelson, T., Wiegand, R. and Brutlag, D. (1981) Ribonucleic acid and other polyanions facilitate chromatin assembly *in vitro.* Biochemistry 20, 2594–2601.
13 Loyola, A. and Almouzni, G. (2004) Histone chaperones, a supporting role in the limelight. Biochim. Biophys. Acta 1677, 3–11.
14 Keller, W. and Wendel, I. (1975) Stepwise relaxation of supercoiled SV40 DNA. Cold Spring Harb. Symp. Quant. Biol. 39, 199–208.
15 Germond, J. E., Hirt, B., Oudet, P., Gross-Bellark, M. and Chambon, P. (1975) Folding of the DNA double helix in chromatin-like structures from simian virus 40. Proc. Natl. Acad. Sci. USA 72, 1843–1847.
16 Hewish, D. R. and Burgoyne, L. A. (1973) Chromatin substructure. The digestion of chromatin DNA at regularly spaced sites by a nuclear deoxyribonuclease. Biochem. Biophys. Res. Commun. 52, 504–510.
17 Noll, M. (1974) Subunit structure of chromatin. Nature 251, 249–251.
18 Olins, A. L. and Olins, D. E. (1974) Spheroid chromatin units (ν bodies). Science 183, 330–332.
19 Tagami, H., Ray-Gallet, D., Almouzni, G. and Nakatani, Y. (2004) Histone H3.1 and H3.3 complexes mediate nucleosome assembly pathways dependent or independent of DNA synthesis. Cell 116, 51–61.
20 Mizuguchi, G., Shen, X., Landry, J., Wu, W. H., Sen, S. and Wu, C. (2004) ATP-Driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. Science 303, 343–348.
21 Frehlick, L. J., Eirín-López, J. M. and Ausió, J. (2007) New insights into the nucleophosmin/nucleoplasmin family of nuclear chaperones. Bioessays 29, 49–59.
22 Kang, Y. J., Olson, M. O., J. and Busch, H. (1974) Phosphorylation of acid-soluble proteins in isolated nucleoli of Novikoff hepatoma ascites cells. Effects of divalent cations. J. Biol. Chem. 249, 5580–5585.
23 Prestayko, A. W., Olson, M. O., J. and Busch, H. (1974) Phosphorylation of proteins of ribosomes and nucleolar preribosomal particles *in vivo* in Novikoff hepatoma ascites cells. FEBS Lett. 44, 131–135.
24 Okuwaki, M., Matsumoto, K., Tsujimoto, M. and Nagata, K. (2001) Function of nucleophosmin/B23, a nucleolar acidic protein, as a histone chaperone. FEBS Lett. 506, 272–276.
25 MacArthur, C. A., Shankar, D. B. and Shackleford, G. M. (1995) *Fgf-8*, activated by proviral insertion, cooperates with the *Wnt-1* transgene in murine mammary tumorigenesis. J. Virol. 69, 2501–2507.
26 MacArthur, C. A. and Shackleford, G. M. (1997) *Npm3:* A novel, widely expressed gene encoding a protein related to the molecular chaperones nucleoplasmin and nucleophosmin. Genomics 42, 137–140.
27 Huang, N., Negi, S., Szebeni, A. and Olson, M. O. J. (2005) Protein NPM3 interacts with the multifunctional nucleolar protein B23/nucleophosmin and inhibits ribosome biogenesis. J. Biol. Chem. 280, 5496–5502.
28 McLay, D. W. and Clarke, H. J. (2003) Remodelling the paternal chromatin at fertilization in mammals. Reproduction 125, 625–633.
29 Ito, T., Tyler, J. K., Bulger, M., Kobayashi, R. and Kadonaga, J. T. (1996) ATP-facilitated chromatin assembly with a nucleoplasmin-like protein from *Drosophila melanogaster.* J. Biol. Chem. 271, 25041–25048.
30 Salvany, L., Chiva, M., Arnan, C., Ausió, J., Subirana, J. A. and Saperas, N. (2004) Mutation of the small acidic tract A1 drastically reduces nucleoplasmin activity. FEBS Lett. 576, 353–357.
31 Swaminathan, V., Kishore, A. H., Febitha, K. K. and Kundu, T. K. (2005) Human histone chaperone nucleophosmin enhances acetylation-dependent chromatin transcription. Mol. Cell. Biol. 25, 7534–7545.
32 Dingwall, C., Dilworth, S. M., Black, S. J., Kearsey, S. E., Cox, L. S. and Laskey, R. A. (1987) Nucleoplasmin cDNA sequence reveals polyglutamic acid tracts and a cluster of sequences homologous to putative nuclear localization signals. EMBO J. 6, 69–74.
33 Hingorani, K., Szebeni, A. and Olson, M. O. J. (2000) Mapping the functional domains of nucleolar protein B23. J. Biol. Chem. 275, 24451–24457.

34 Dutta, S., Akey, I. V., Dingwall, C., Hartman, K. L., Laue, T., Nolte, R. T., Head, J. F. and Akey, C. W. (2001) The crystal structure of nucleoplasmin-core: Implications for histone binding and nucleosome assembly. Mol. Cell 8, 841–853.
35 Namboodiri, V. M., H., Dutta, S., Akey, I. V., Head, J. F. and Akey, C. W. (2003) The crystal structure of *Drosophila* NLP-core provides insight into pentamer formation and histone binding. Structure 11, 175–186.
36 Namboodiri, V. M., H., Akey, I. V., Schmidt-Zachmann, M. S., Head, J. F. and Akey, C. W. (2004) The structure and function of *Xenopus* NO38-core, a histone chaperone in the nucleolus. Structure 12, 2149–2160.
37 Zirwes, R. F., Schmidt-Zachmann, M. S. and Franke, W. W. (1997) Identification of a small, very acidic constitutive nucleolar protein (NO29) as a member of the nucleoplasmin family. Proc. Natl. Acad. Sci. USA 94, 11387–11392.
38 Kleinschmidt, J. A. and Franke, W. W. (1982) Soluble acidic complexes containing histones H3 and H4 in nuclei of *Xenopus laevis* oocytes. Cell 29, 799–809.
39 Kleinschmidt, J. A., Fortkamp, E., Krohne, G., Zentgraf, H. and Franke, W. W. (1985) Co-existence of two different types of soluble histone complexes in nuclei of *Xenopus laevis* oocytes. J. Biol. Chem. 260, 1166–1176.
40 Kleinschmidt, J. A., Seiter, A. and Zentgraf, H. (1990) Nucleosome assembly *in vitro:* Separate histone transfer and synergistic interaction of native histone complexes purified from nuclei of *Xenopus laevis* oocytes. EMBO J. 9, 1309–1318.
41 Cotten, M., Sealy, L. and Chalkley, R. (1986) Massive phosphorylation distinguishes *Xenopus laevis* nucleoplasmin isolated from oocytes or unfertilized eggs. Biochemistry 25, 5063–5069.
42 Lu, Z., Zhang, C. and Zhai, Z. (2005) Nucleoplasmin regulates chromatin condensation during apoptosis. Proc. Natl. Acad. Sci. USA 102, 2778–2783.
43 Okuda, M., Horn, H. F., Tarapore, P., Tokuyama, Y., Smulian, A. G., Chan, P. K., Knudsen, E. S., Hofmann, I. A., Snyder, J. D., Bove, K. E. and Fukasawa, K. (2000) Nucleophosmin/B23 is a target of CDK2/Cyclin E in centrosome duplication. Cell 103, 127–140.
44 Okuwaki, M., Tsujimoto, M. and Nagata, K. (2002) The RNA binding activity of a ribosome biogenesis factor, nucleophosmin/B23, is modulated by phosphorylation with a cell cycle-dependent kinase and by association with its subtype. Mol. Biol. Cell 13, 2016–2030.
45 Tarapore, P., Shinmura, K., Suzuki, H., Tokuyama, Y., Kim, S. H., Mayeda, A. and Fukasawa, K. (2006) Thr^{199} phosphorylation targets nucleophosmin to nuclear speckles and represses pre-mRNA processing. FEBS Lett. 580, 399–409.
46 Liu, X., Liu, Z., Jang, S. W., Ma, Z., Shinmura, K., Kang, S., Dong, S., Chen, J., Fukasawa, K. and Ye, K. (2007) Sumoylation of nucleophosmin/B23 regulates its subcellular localization, mediating cell proliferation and survival. Proc. Natl. Acad. Sci. USA 104, 9679–9684.
47 Ju, B. G., Solum, D., Song, E. J., Lee, K. J., Rose, D. W., Glass, C. K. and Rosenfeld, M. G. (2004) Activating the PARP-1 sensor component of the Groucho/TLE1 corepressor complex mediates a CaMkinase IIδ-dependent neurogenic gene activation pathway. Cell 119, 815–829.
48 Regnard, C., Desbruyères, E., Huet, J. C., Beauvallet, C., Pernollet, J. C. and Eddé, B. (2000) Polyglutamylation of nucleosome assembly proteins. J. Biol. Chem. 275, 15969–15976.
49 Regnard, C., Fesquet, D., Janke, C., Boucher, D., Desbruyères, E., Koulakoff, A., Insina, C., Travo, P. and Eddé, B. (2003) Characterisation of PGs1, a subunit of a protein complex co-purifying with tubulin polyglutamylase. J. Cell Sci. 116, 4181–4190.
50 Bonner, W. M. (1975) Protein migration into nuclei. II. Frog oocyte nuclei accumulate a class of microinjected oocyte nuclear proteins and exclude a class of microinjected oocyte cytoplasmic proteins. J. Cell Biol. 64, 431–437.
51 De Robertis, E. M., Longthorne, R. F. and Gurdon, J. B. (1978) Intracellular migration of nuclear proteins in *Xenopus* oocytes. Nature 272, 254–256.
52 Kleinschmidt, J., Dingwall, C., Maier, G. and Franke, W. W. (1986) Molecular characterization of a karyophilic, histone-binding protein: cDNA cloning, amino acid sequence and expression of nuclear protein N1/N2 of *Xenopus laevis.* EMBO J. 5, 3547–3552.
53 Welch, J. E., Zimmerman, L. J., Joseph, D. R. and O'Rand, M. G. (1990) Characterization of a sperm-specific nuclear autoantigenic protein. I. complete sequence and homology with the *Xenopus* protein, N1/N2. Biol. Reprod. 43, 559–568.
54 O'Rand, M. G., Richardson, R. T., Zimmerman, L. J. and Widgren, E. E. (1992) Sequence and localization of human NASP: Conservation of a *Xenopus* histone-binding protein. Dev. Biol. 154, 37–44.
55 Poveda, A., Pamblanco, M., Tafrov, S., Tordera, V., Sternglanz, R. and Sendra, R. (2004) Hif1 is a component of yeast histone acetyltransferase B, a complex mainly localized in the nucleus. J. Biol. Chem. 279, 16033–16043.
56 Ai, X. and Parthun, M. R. (2004) The nuclear Hat1p/Hat2p complex: A molecular link between type B histone acetyltransferases and chromatin assembly. Mol. Cell 14, 195–205.
57 Kleinschmidt, J. A. and Seiter, A. (1988) Identification of domains involved in nuclear uptake and histone binding of protein N1 of *Xenopus laevis.* EMBO J. 7, 1605–1614.
58 Batova, I. and O'Rand, M. G. (1996) Histone-binding domains in a human nuclear autoantigenic sperm protein. Biol. Reprod. 54, 1238–1244.
59 Richardson, R. T., Batova, I. N., Widgren, E. E., Zheng, L. X., Whitfield, M., Marzluff, W. F. and O'Rand, M. G. (2000) Characterization of the histone H1-binding protein, NASP, as a cell cycle-regulated somatic protein. J. Biol. Chem. 275, 30378–30386.
60 Dabauvalle, M. C. and Franke, W. W. (1982) Karyophilic proteins: Polypeptides synthesized *in vitro* accumulate in the nucleus on microinjection into the cytoplasm of amphibian oocytes. Proc. Natl. Acad. Sci. USA 79, 5302–5306.
61 Dilworth, S. M., Black, S. J. and Laskey, R. A. (1987) Two complexes that contain histones are required for nucleosome assembly *in vitro:* Role of nucleoplasmin and N1 in *Xenopus* egg extracts Cell 51, 1009–1018.
62 Alekseev, O. M., Bencic, D. C., Richardson, R. T., Widgren, E. E. and O'Rand, M. G. (2003) Overexpression of the linker histone-binding protein tNASP affects progression through the cell cycle. J. Biol. Chem. 278, 8846–8852.
63 Alekseev, O. M., Widgren, E. E., Richardson, R. T. and O'Rand, M. G. (2005) Association of NASP with HSP90 in mouse spermatogenic cells: Stimulation of ATPase activity and transport of linker histones into nuclei. J. Biol. Chem. 280, 2904–2911.
64 Alekseev, O. M., Richardson, R. T., Pope, M. R. and O'Rand, M. G. (2005) Mass spectrometry identification of NASP binding partners in HeLa cells. Proteins 61, 1–5.
65 Weinmann, A. S., Yan, P. S., Oberley, M. J., Huang, T. H.-M. and Farnham, P. J. (2002) Isolating human transcription factor targets by coupling chromatin immunoprecipitation and CpG island microarray analysis. Genes Dev. 16, 235–244.
66 Richardson, R. T., Bencic, D. C. and O'Rand, M. G. (2001) Comparison of mouse and human NASP genes and expression in human transformed and tumor cell lines. Gene 274, 67–75.
67 Richardson, R. T., Alekseev, O., Alekseev, O. M. and O'Rand, M. G. (2006) Characterization of the NASP promoter in 3T3 fibroblasts and mouse spermatogenic cells. Gene 371, 52–58.
68 Welch, J. E. and O'Rand, M. G. (1990) Characterization of a sperm-specific nuclear autoantigenic protein. II. expression and localization in the testis. Biol. Reprod. 43, 569–578.
69 Richardson, R. T., Alekseev, O. M., Grossman, G., Widgren, E. E., Thresher, R., Wagner, E. J., Sullivan, K. D., Marzluff, W. F. and O'Rand, M. G. (2006) Nuclear autoantigenic sperm

protein (NASP), a linker histone chaperone that is required for cell proliferation. J. Biol. Chem. 281, 21526–21534.
70 Winston, F., Chaleff, D. T., Valent, B. and Fink, G. R. (1984) Mutations affecting Ty-mediated expression of the *HIS4* gene of *Saccharomyces cerevisiae.* Genetics 107, 179–197.
71 Denis, C. L. (1984) Identification of new genes involved in the regulation of yeast alcohol dehydrogenase II. Genetics 108, 833–844.
72 Neigeborn, L., Rubin, K. and Carlson, M. (1986) Suppressors of *snf2* mutations restore invertase derepression and cause temperature-sensitive lethality in yeast. Genetics 112, 741–753.
73 Neigeborn, L., Celenza, J. L. and Carlson, M. (1987) *SSN20* is an essential gene with mutant alleles that suppress defects in *SUC2* transcription in *Saccharomyces cerevisiae.* Mol. Cell. Biol. 7, 672–678.
74 Clark-Adams, C. D. and Winston, F. (1987) The *SPT6* gene is essential for growth and is required for δ-mediated transcription in *Saccharomyces cerevisiae.* Mol. Cell. Biol. 7, 679–686.
75 Denis, C. L. and Malvar, T. (1990) The *CCR4* gene from *Saccharomyces cerevisiae* is required for both nonfermentative and *spt*-mediated gene expression. Genetics 124, 283–291.
76 Swanson, M. S., Carlson, M. and Winston, F. (1990) *SPT6*, an essential gene that affects transcription in *Saccharomyces cerevisiae*, encodes a nuclear protein with an extremely acidic amino terminus. Mol. Cell. Biol. 10, 4935–4941.
77 Nishiwaki, K., Sano, T. and Miwa, J. (1993) *emb-5*, a gene required for the correct timing of gut precursor cell division during gastrulation in *Caenorhabditis elegans*, encodes a protein similar to the yeast nuclear protein SPT6. Mol. Gen. Genet. 239, 313–322.
78 Chiang, P. W., Wang, S., Smithivas, P., Song, W. J., Ramamoorthy, S., Hillman, J., Puett, S., Van Keuren, M. L., Crombez, E., Kumar, A., Glover, T. W., Miller, D. E. et al. (1996) Identification and analysis of the human and murine putative chromatin structure regulator SUPT6H and *Supt6h*. Genomics 34, 328–333.
79 Bortvin, A. and Winston, F. (1996) Evidence that Spt6p controls chromatin structure by a direct interaction with histones. Science 272, 1473–1476.
80 Maclennan, A. J. and Shaw, G. (1993) A yeast SH2 domain. Trends Biochem. Sci. 18, 464–465.
81 Doherty, A. J., Serpell, L. C. and Ponting, C. P. (1996) The helix-hairpin-helix DNA-binding motif: A structural basis for non-sequence-specific recognition of DNA. Nucleic Acids Res. 24, 2488–2497.
82 Ponting, C. P. (2002) Novel domains and orthologues of eukaryotic transcription elongation factors. Nucleic Acids Res. 30, 3643–3652.
83 Compagnone-Post, P. A. and Osley, M. A. (1996) Mutations in the *SPT4, SPT5*, and *SPT6* genes alter transcription of a subset of histone genes in *Saccharomyces cerevisiae.* Genetics 143, 1543–1554.
84 Adkins, M. W. and Tyler, J. K. (2006) Transcriptional activators are dispensable for transcription in the absence of Spt6-mediated chromatin reassembly of promoter regions. Mol. Cell 21, 405–416.
85 Kaplan, C. D., Laprade, L. and Winston, F. (2003) Transcription elongation factors repress transcription initiation from cryptic sites. Science 301, 1096–1099.
86 Hartzog, G. A., Wada, T., Handa, H. and Winston, F. (1998) Evidence that Spt4, Spt5, and Spt6, control transcription elongation by RNA polymerase II in *Saccharomyces cerevisiae.* Genes Dev. 12, 357–369.
87 Swanson, M. S. and Winston, F. (1992) *SPT4*, *SPT5* and *SPT6* interactions: Effects on transcription and viability in *Saccharomyces cerevisiae.* Genetics 132, 325–336.
88 Krogan, N. J., Kim, M., Ahn, S. H., Zhong, G., Kobor, M. S., Cagney, G., Emili, A., Shilatifard, A., Buratowski, S. and Greenblatt, J. F. (2002) RNA polymerase II elongation factors of *Saccharomyces cerevisiae:* A targeted proteomnics approach. Mol. Cell. Biol. 22, 6979–6992.
89 Kaplan, C. D., Morris, J. R., Wu, C. and Winston, F. (2000) Spt5 and spt6 are associated with active transcription and have characteristics of general elongation factors in *D. melanogaster.* Genes Dev. 14, 2623–2634.
90 Andrulis, E. D., Guzmán, E., Döring, P., Werner, J. and Lis, J. T. (2000) High-resolution localization of *Drosophila* Spt5 and Spt6 at heat shock genes *in vivo:* Roles in promoter proximal pausing and transcription elongation. Genes Dev. 14, 2635–2649.
91 Saunders, A., Werner, J., Andrulis, E. D., Nakayama, T., Hirose, S., Reinberg, D. and Lis, J. T. (2003) Tracking FACT and the RNA polymerase II elongation complex through chromatin *in vivo.* Science 301, 1094–1096.
92 Yoh, S. M., Cho, H., Pickle, L., Evans, R. M. and Jones, K. A. (2007) The Spt6 SH2 domain binds Ser2-P RNAPII to direct Iws1-dependent mRNA splicing and export. Genes Dev 21, 160–174.
93 Kim, M., Ahn, S. H., Krogan, N. J., Greenblatt, J. F. and Buratowski, S. (2004) Transitions in RNA polymerase II elongation complexes at the 3′ ends of genes. EMBO J. 23, 354–364.
94 Kaplan, C. D., Holland, M. J. and Winston, F. (2005) Interaction between transcription elongation factors and mRNA 3′-end formation at the *Saccharomyces cerevisiae GAL10-GAL7* locus. J. Biol. Chem. 280, 913–922.
95 Andrulis, E. D., Werner, J., Nazarian, A., Erdjument-Bromage, H., Tempst, P. and Lis, J. T. (2002) The RNA processing exosome is linked to elongating RNA polymerase II in *Drosophila.* Nature 420, 837–841.
96 Ishimi, Y., Yasuda, H., Hirosumi, J., Hanaoka, F. and Yamada, M. (1983) A protein which facilitates assembly of nucleosome-like structures *in vitro* in mammalian cells. J. Biochem. (Tokyo) 94, 735–744.
97 Ishimi, Y., Hirosumi, J., Sato, W., Sugasawa, K., Yokota, S., Hanaoka, F. and Yamada, M. (1984) Purification and initial characterization of a protein which facilitates assembly of nucleosome-like structure from mammalian cells. Eur. J. Biochem. 142, 431–439.
98 Wongwisansri, S. and Laybourn, P. J. (2004) Reconstitution of yeast chromatin using yNap1p. Methods Enzymol. 375, 103–117.
99 Ishimi, Y. and Kikuchi, A. (1991) Identification and molecular cloning of yeast homolog of nucleosome assembly protein I which facilitates nucleosome assembly *in vitro.* J. Biol. Chem. 266, 7025–7029.
100 Simon, H. U., Mills, G. B., Kozlowski, M., Hogg, D., Branch, D., Ishimi, Y. and Siminovitch, K. A. (1994) Molecular characterization of hNRP, a cDNA encoding a human nucleosome-assembly-protein-I-related gene product involved in the induction of cell proliferation. Biochem. J. 297, 389–397.
101 Yoon, H. W., Kim, M. C., Lee, S. Y., Hwang, I., Bahk, J. D., Hong, J. C., Ishimi, Y. and Cho, M. J. (1995) Molecular cloning and functional characterization of a cDNA encoding nucleosome assembly protein 1 (NAP-1) from soybean. Mol. Gen. Genet. 249, 465–473.
102 Ito, T., Bulger, M., Kobayashi, R. and Kadonaga, J. T. (1996) *Drosophila* NAP-1 is a core histone chaperone that functions in ATP-facilitated assembly of regularly spaced nucleosomal arrays. Mol. Cell. Biol. 16, 3112–3124.
103 Dong, A., Zhu, Y., Yu, Y., Cao, K., Sun, C. and Shen, W. H. (2003) Regulation of biosynthesis and intracellular localization of rice and tobacco homologues of nucleosome assembly protein 1. Planta 216, 561–570.
104 Steer, W. M., Abu-Daya, A., Brickwood, S. J., Mumford, K. L., Jordanaires, N., Mitchell, J., Robinson, C., Thome, A. W. and Guille, M. J. (2003) *Xenopus* nucleosome assembly protein becomes tissue-restricted during development and can alter the expression of specific genes. Mech. Dev. 120, 1045–1057.

105 Fujii-Nakata, T., Ishimi, Y., Okuda, A. and Kikuchi, A. (1992) Functional analysis of nucleosome assembly protein, NAP-1. The negatively charged COOH-terminal region is not necessary for the intrinsic assembly activity. J. Biol. Chem. 267, 20980–20986.
106 Park, Y. J. and Luger, K. (2006) The structure of nucleosome assembly protein 1. Proc. Natl. Acad. Sci. USA 103, 1248–1253.
107 Kawase, H., Okuwaki, M., Miyaji, M., Ohba, R., Handa, H., Ishimi, Y., Fujii-Nakata, T., Kikuchi, A. and Nagata, K. (1996) NAP-I is a functional homologue of TAF-I that is required for replication and transcription of the adenovirus genome in a chromatin-like structure. Genes Cells 1, 1045–1056.
108 Rodriguez, P., Munroe, D., Prawitt, D., Chu, L. L., Bric, E., Kim, J., Reid, L. H., Davies, C., Nakagama, H., Loebbert, R., Winterpacht, A., Petruzzi, M. J. et al. (1997) Functional characterization of human nucleosome assembly protein-2 (NAP1L4) suggests a role as a histone chaperone. Genomics 44, 253–265.
109 Shen, H. H., Huang, A. M., Hoheisel, J. and Tsai, S. F. (2001) Identification and characterization of a SET/NAP protein encoded by a brain-specific gene, MB20. Genomics 71, 21–33.
110 Wang, G. S., Hong, C. J., Yen, T. Y., Huang, H. Y., Ou, Y., Huang, T. N., Jung, W. G., Kuo, T. Y., Sheng, M., Wang, T. F. and Hsueh, Y. P. (2004) Transcriptional modification by a CASK-interacting nucleosome assembly protein. Neuron 42, 113–128.
111 Park, Y. J., Chodaparambil, J. V., Bao, Y. H., McBryant, S. J. and Luger, K. (2005) Nucleosome assembly protein 1 exchanges histone H2A-H2B dimers and assists nucleosome sliding. J. Biol. Chem. 280, 1817–1825.
112 Dong, A., Liu, Z., Zhu, Y., Yu, F., Li, Z., Cao, K. and Shen, W. H. (2005) Interacting proteins and differences in nuclear transport reveal specific functions for the NAP1 family proteins in plants. Plant Physiol. 138, 1446–1456.
113 Kepert, J. F., Mazurkiewicz, J., Heuvelman, G. L., Tóth, K. F. and Rippe, K. (2005) NAP1 modulates binding of linker histone H1 to chromatin and induces an extended chromatin fiber conformation. J. Biol. Chem. 280, 34063–34072.
114 Shintomi, K., Iwabuchi, M., Saeki, H., Ura, K., Kishimoto, T. and Ohsumi, K. (2005) Nucleosome assembly protein-1 is a linker histone chaperone in *Xenopus* eggs. Proc. Natl. Acad. Sci. USA 102, 8210–8215.
115 Saeki, H., Ohsumi, K., Aihara, H., Ito, T., Hirose, S., Ura, K. and Kaneda, Y. (2005) Linker histone variants control chromatin dynamics during early embryogenesis. Proc. Natl. Acad. Sci. USA 102, 5697–5702.
116 Lankenau, S., Barnickel, T., Marhold, J., Lyko, F., Mechler, B. M. and Lankenau, D. H. (2003) Knock-out targeting of the *Drosophila Nap1* gene and examination of DNA repair tracts in the recombination products. Genetics 163, 611–623.
117 Li, M. F., Strand, D., Krehan, A., Pyerin, W., Heid, H., Neumann, B. and Mechler, B. M. (1999) Casein kinase 2 binds and phosphorylates the nucleosome assembly protein-1 (NAP1) in *Drosophila melanogaster.* J. Mol. Biol. 293, 1067–1084.
118 Rodriguez, P., Pelletier, J., Price, G. B. and Zannis-Hadjopoulos, M. (2000) NAP-2: Histone chaperone function and phosphorylation state through the cell cycle. J. Mol. Biol. 298, 225–238.
119 Mosammaparast, N., Ewart, C. S. and Pemberton, L. F. (2002) A role for nucleosome assembly protein 1 in the nuclear transport of histones H2A and H2B. EMBO J. 21, 6527–6538.
120 Miyaji-Yamaguchi, M., Kato, K., Nakano, R., Akashi, T., Kikuchi, A. and Nagata, K. (2003) Involvement of nucleocytoplasmic shuttling of yeast Nap1 in mitotic progression. Mol. Cell. Biol. 23, 6672–6684.
121 Dingwall, C., Robbins, J., Dilworth, S. M., Roberts, B. and Richardson, W. D. (1988) The nucleoplasmin nuclear location sequence is larger and more complex than that of SV-40 large T antigen. J. Cell Biol. 107, 841–849.
122 Kalderon, D., Richardson, W. D., Markham, A. F. and Smith, A. E. (1984) Sequence requirements for nuclear location of simian virus 40 large-T antigen. Nature 311, 33–38.
123 Robbins, J., Dilworth, S. M., Laskey, R. A. and Dingwall, C. (1991) Two interdependent basic domains in nucleoplasmin nuclear targeting sequence: Identification of a class of bipartite nuclear targeting sequence. Cell 64, 615–623.
124 Okuwaki, M., Kato, K., Shimahara, H., Tate, S. and Nagata, K. (2005) Assembly and disassembly of nucleosome core particles containing histone variants by human nucleosome assembly protein I. Mol. Cell. Biol. 25, 10639–10651.
125 Park, Y. J. and Luger, K. (2006) Structure and function of nucleosome assembly proteins. Biochem. Cell Biol. 84, 549–558.
126 Rehtanz, M., Schmidt, H. M., Warthorst, U. and Steger, G. (2004) Direct interaction between nucleosome assembly protein 1 and the papillomavirus E2 proteins involved in activation of transcription. Mol. Cell. Biol. 24, 2153–2168.
127 von Lindern, M., van Baal, S., Wiegant, J., Raap, A., Hagemeijer, A. and Grosveld, G. (1992) *can*, a putative oncogene associated with myeloid leukemogenesis, may be activated by fusion of its 3′ half to different genes: Characterization of the *set* gene. Mol. Cell. Biol. 12, 3346–3355.
128 Matsumoto, K., Nagata, K., Ui, M. and Hanaoka, F. (1993) Template activating factor I, a novel host factor required to stimulate the adenovirus core DNA replication. J. Biol. Chem. 268, 10582–10587.
129 Nagata, K., Kawase, H., Handa, H., Yano, K., Yamasaki, M., Ishimi, Y., Okuda, A., Kikuchi, A. and Matsumoto, K. (1995) Replication factor encoded by a putative oncogene, *set*, associated with myeloid leukemogenesis. Proc. Natl. Acad. Sci. USA 92, 4279–4283.
130 Wen, C., Levitan, D., Li, X. and Greenwald, I. (2000) *spr-2*, a suppressor of the egg-laying defect caused by loss of *sel-12* presenilin in *Caenorhabditis elegans*, is a member of the SET protein subfamily. Proc. Natl. Acad. Sci. USA 97, 14524–14529.
131 Selth, L. and Svejstrup, J. Q. (2007) Vps75, a new yeast member of the NAP histone chaperone family. J. Biol. Chem. 282, 12358–12362.
132 Tsubota, T., Berndsen, C. E., Erkmann, J. A., Smith, C. L., Yang, L., Freitas, M. A., Denu, J. M. and Kaufman, P. D. (2007) Histone H3-K56 acetylation is catalyzed by histone chaperone-dependent complexes. Mol. Cell 25, 703–712.
133 Matsumoto, K., Nagata, K., Miyaji-Yamaguchi, M., Kikuchi, A. and Tsujimoto, M. (1999) Sperm chromatin decondensation by template activating factor I through direct interaction with basic proteins. Mol. Cell. Biol. 19, 6940–6952.
134 Muto, S., Senda, M., Akai, Y., Sato, L., Suzuki, T., Nagai, R., Senda, T. and Horikoshi, M. (2007) Relationship between the structure of SET/TAF-Iβ/INHAT and its histone chaperone activity. Proc. Natl. Acad. Sci. USA 104, 4285–4290.
135 Seo, S. B., McNamara, P., Heo, S., Turner, A., Lane, W. S. and Chakravarti, D. (2001) Regulation of histone acetylation and transcription by INHAT, a human cellular complex containing the set oncoprotein. Cell 104, 119–130.
136 Okuwaki, M. and Nagata, K. (1998) Template activating factor-I remodels the chromatin structure and stimulates transcription from the chromatin template. J. Biol. Chem. 273, 34511–34518.
137 Seo, S. B., Macfarlan, T., McNamara, P., Hong, R., Mukai, Y., Heo, S. and Chakravarti, D. (2002) Regulation of histone acetylation and transcription by nuclear protein pp32, a subunit of the INHAT complex. J. Biol. Chem. 277, 14005–14010.
138 Kutney, S. N., Hong, R., Macfarlan, T. and Chakravarti, D. (2004) A signaling role of histone-binding proteins and INHAT subunits pp32 and Set/TAF-Iβ in integrating chromatin hypoacetylation and transcriptional repression. J. Biol. Chem. 279, 30850–30855.

139 Schneider, R., Bannister, A. J., Weise, C. and Kouzarides, T. (2004) Direct binding of INHAT to H3 tails disrupted by modifications. J. Biol. Chem. 279, 23859–23862.

140 Miyamoto, S., Suzuki, T., Muto, S., Aizawa, K., Kimura, A., Mizuno, Y., Nagino, T., Imai, Y., Adachi, N., Horikoshi, M. and Nagai, R. (2003) Positive and negative regulation of the cardiovascular transcription factor KLF5 by p300 and the oncogenic regulator SET through interaction and acetylation on the DNA-binding domain. Mol. Cell. Biol. 23, 8528–8541.

141 Suzuki, T., Muto, S., Miyamoto, S., Aizawa, K., Horikoshi, M. and Nagai, R. (2003) Functional interaction of the DNA-binding transcription factor Sp1 through its DNA-binding domain with the histone chaperone TAF-I. J. Biol. Chem. 278, 28758–28764.

142 Loven, M. A., Muster, N., Yates, J. R. and Nardulli, A. M. (2003) A novel estrogen receptor α-associated protein, template-activating factor Iβ, inhibits acetylation and transactivation. Mol. Endocrinol. 17, 67–78.

143 Compagnone, N. A., Zhang, P., Vigne, J. L. and Mellon, S. H. (2000) Novel role for the nuclear phosphoprotein SET in transcriptional activation of P450c17 and initiation of neurosteroidogenesis. Mol. Endocrinol. 14, 875–888.

144 Telese, F., Bruni, P., Donizetti, A., Gianni, D., D'Ambrosio, C., Scaloni, A., Zambrano, N., Rosenfeld, M. G. and Russo, T. (2005) Transcription regulation by the adaptor protein Fe65 and the nucleosome assembly factor SET. EMBO Rep. 6, 77–82.

145 Kraemer, D., Wozniak, R. W., Blobel, G. and Radu, A. (1994) The human CAN protein, a putative oncogene product associated with myeloid leukemogenesis, is a nuclear pore complex protein that faces the cytoplasm. Proc. Natl. Acad. Sci. USA 91, 1519–1523.

146 Beresford, P. J., Kam, C. M., Powers, J. C. and Lieberman, J. (1997) Recombinant human granzyme A binds to two putative HLA-associated proteins and cleaves one of them. Proc. Natl. Acad. Sci. USA 94, 9285–9290.

147 Zhang, D., Pasternack, M. S., Beresford, P. J., Wagner, L., Greenberg, A. H. and Lieberman, J. (2001) Induction of rapid histone degradation by the cytotoxic T lymphocyte protease granzyme A. J. Biol. Chem. 276, 3683–3690.

148 Fan, Z., Beresford, P. J., Zhang, D. and Lieberman, J. (2002) HMG2 interacts with the nucleosome assembly protein SET and is a target of the cytotoxic T-lymphocyte protease granzyme A. Mol. Cell. Biol. 22, 2810–2820.

149 Fan, Z., Beresford, P. J., Oh, D. Y., Zhang, D. and Lieberman, J. (2003) Tumor suppressor NM23-H1 is a granzyme A-activated DNase during CTL-mediated apoptosis, and the nucleosome assembly protein SET is its inhibitor. Cell 112, 659–672.

150 Estanyol, J. M., Jaumot, M., Casanovas, O., Rodriguez-Vilarrupla, A., Agell, N. and Bachs, O. (1999) The protein SET regulates the inhibitory effect of p21(Cip1) cyclin E-cyclin-dependent kinase 2 activity. J. Biol. Chem. 274, 33161–33165.

151 Canela, N., Rodriguez-Vilarrupla, A., Estanyol, J. M., Diaz, C., Pujol, M. J., Agell, N. and Bachs, O. (2003) The SET protein regulates G_2/M transition by modulating cyclin B-cyclin-dependent kinase 1 activity. J. Biol. Chem. 278, 1158–1164.

152 Shimoyama, T., Kato, K., Miyaji-Yamaguchi, M. and Nagata, K. (2005) Synergistic action of MLL, a TRX protein with template activating factor-I, a histone chaperone. FEBS Lett. 579, 757–762.

153 Stillman, B. (1986) Chromatin assembly during SV40 DNA replication *in vitro.* Cell 45, 555–565.

154 Smith, S. and Stillman, B. (1989) Purification and characterization of CAF-I, a human cell factor required for chromatin assembly during DNA replication *in vitro.* Cell 58, 15–25.

155 Kaufman, P. D., Kobayashi, R., Kessler, N. and Stillman, B. (1995) The p150 and p60 subunits of chromatin assembly factor I: A molecular link between newly synthesized histones and DNA replication. Cell 81, 1105–1114.

156 Kamakaka, R. T., Bulger, M., Kaufman, P. D., Stillman, B. and Kadonaga, J. T. (1996) Postreplicative chromatin assembly by *Drosophila* and human chromatin assembly factor 1. Mol. Cell. Biol. 16, 810–817.

157 Tyler, J. K., Bulger, M., Kamakaka, R. T., Kobayashi, R. and Kadonaga, J. T. (1996) The p55 subunit of *Drosophila* chromatin assembly factor 1 is homologous to a histone deacetylase-associated protein. Mol. Cell. Biol. 16, 6149–6159.

158 Tyler, J. K., Collins, K. A., Prasad-Sinha, J., Amiott, E., Bulger, M., Harte, P. J., Kobayashi, R. and Kadonaga, J. T. (2001) Interaction between the *Drosophila* CAF-1 and ASF1 chromatin assembly factors. Mol. Cell. Biol. 21, 6574–6584.

159 Kaufman, P. D., Kobayashi, R. and Stillman, B. (1997) Ultraviolet radiation sensitivity and reduction of telomeric silencing *Saccharomyces cerevisiae* cells lacking chromatin assembly factor-I. Genes Dev. 11, 345–357.

160 Enomoto, S., McCune-Zierath, P. D., Gerami-Nejad, M., Sanders, M. A. and Berman, J. (1997) *RLF2*, a subunit of yeast chromatin assembly factor-I, is required for telomeric chromatin function *in vivo.* Genes Dev. 11, 358–370.

161 Verreault, A., Kaufman, P. D., Kobayashi, R. and Stillman, B. (1996) Nucleosome assembly by a complex of CAF-1 and acetylated histones H3/H4. Cell 87, 95–104.

162 Quivy, J. P., Grandi, P. and Almouzni, G. (2001) Dimerization of the largest subunit of chromatin assembly factor 1: Importance *in vitro* and during *Xenopus* early development. EMBO J. 20, 2015–2027.

163 Shibahara, K. and Stillman, B. (1999) Replication-dependent marking of DNA by PCNA facilitates CAF-1-coupled inheritance of chromatin. Cell 96, 575–585.

164 Moggs, J. G., Grandi, P., Quivy, J. P., Jónsson, Z. O., Hübscher, U., Becker, P. B. and Almouzni, G. (2000) A CAF-1-PCNA-mediated chromatin assembly pathway triggered by sensing DNA damage. Mol. Cell. Biol. 20, 1206–1218.

165 Krude, T. (1995) Chromatin assembly factor 1 (CAF-1) colocalizes with replication foci in HeLa cell nuclei. Exp. Cell Res. 220, 304–311.

166 Marheineke, K. and Krude, T. (1998) Nucleosome assembly activity and intracellular localization of human CAF-1 changes during the cell division cycle. J. Biol. Chem. 273, 15279–15286.

167 Murzina, N., Verreault, A., Laue, E. and Stillman, B. (1999) Heterochromatin dynamics in mouse cells: Interaction between chromatin assembly factor 1 and HP1 proteins. Mol. Cell 4, 529–540.

168 Blouin, J. L., Duriaux-Sail, G., Chen, H., Gos, A., Morris, M. A., Rossier, C. and Antonarakis, S. E. (1996) Mapping of the gene for the p60 subunit of the human chromatin assembly factor (CAF1A) to the Down syndrome region of chromosome 21. Genomics 33, 309–312.

169 Kaufman, P. D., Cohen, J. L. and Osley, M. A. (1998) Hir proteins are required for position-dependent gene silencing in *Saccharomyces cerevisiae* in the absence of chromatin assembly factor I. Mol. Cell. Biol. 18, 4793–4806.

170 Kirov, N., Shtilbans, A. and Rushlow, C. (1998) Isolation and characterization of a new gene encoding a member of the HIRA family of proteins from *Drosophila melanogaster.* Gene 212, 323–332.

171 Tang, Y., Poustovoitov, M. V., Zhao, K., Garfinkel, M., Canutescu, A., Dunbrack, R., Adams, P. D. and Marmorstein, R. (2006) Structure of a human ASF1a-HIRA complex and insights into specificity of histone chaperone complex assembly. Nat. Struct. Mol. Biol. 13, 921–929.

172 Krawitz, D. C., Kama, T. and Kaufman, P. D. (2002) Chromatin assembly factor I mutants defective for PCNA binding require Asf1/Hir proteins for silencing. Mol. Cell. Biol. 22, 614–625.

173 Mello, J. A., Silljé, H. H., W., Roche, D. M., J., Kirschner, D. B., Nigg, E. A. and Almouzni, G. (2002) Human Asf1 and CAF-1 interact and synergize in a repair-coupled nucleosome assembly pathway. EMBO Rep. 3, 329–334.

174 Sanematsu, F., Takami, Y., Barman, H. K., Fukagawa, T., Ono, T., Shibahara, K. and Nakayama, T. (2006) Asf1 is required for viability and chromatin assembly during DNA replication in vertebrate cells. J. Biol. Chem. 281, 13817–13827.
175 Martini, E., Roche, D. M., J., Marheineke, K., Verreault, A. and Almouzni, G. (1998) Recruitment of phosphorylated chromatin assembly factor 1 to chromatin after UV irradiation of human cells. J. Cell Biol. 143, 563–575.
176 Keller, C. and Krude, T. (2000) Requirement of cyclin/Cdk2 and protein phosphatase 1 activity for chromatin assembly factor 1-dependent chromatin assembly during DNA synthesis. J. Biol. Chem. 275, 35512–35521.
177 Smith, S. and Stillman, B. (1991) Stepwise assembly of chromatin during DNA replication *in vitro.* EMBO J. 10, 971–980.
178 Gaillard, P.-H. L., Martini, E. M.-D., Kaufman, P. D., Stillman, B., Moustacchi, E. and Almouzni, G. (1996) Chromatin assembly coupled to DNA repair: A new role for chromatin assembly factor I. Cell 86, 887–896.
179 Ruggieri, R., Tanaka, K., Nakafuku, M., Kaziro, Y., Toh-e, A. and Matsumoto, K. (1989) *MSI1*, a negative regulator of the RAS-cAMP pathway in *Saccharomyces cerevisiae.* Proc. Natl. Acad. Sci. USA 86, 8778–8782.
180 Müller, J., Hart, C. M., Francis, N. J., Vargas, M. L., Sengupta, A., Wild, B., Miller, E. L., O'Connor, M. B., Kingston, R. E. and Simon, J. A. (2002) Histone methyltransferase activity of a *Drosophila* polycomb group repressor complex. Cell 111, 197–208.
181 Martínez-Balbás, M. A., Tsukiyama, T., Gdula, D. and Wu, C. (1998) *Drosophila* NURF-55, a WD repeat protein involved in histone metabolism. Proc. Natl. Acad. Sci. USA 95, 132–137.
182 Groth, A., Rocha, W., Verreault, A. and Almouzni, G. (2007) Chromatin challenges during DNA replication and repair. Cell 128, 721–733.
183 Jiao, R., Harrigan, J. A., Shevelev, I., Dietschy, T., Selak, N., Indig, F. E., Piotrowski, J., Janscak, P., Bohr, V. A. and Stagljar, I. (2007) The Werner syndrome protein is required for recruitment of chromatin assembly factor 1 following DNA damage. Oncogene 26, 3811–3822.
184 Tyler, J. K., Adams, C. R., Chen, S. R., Kobayashi, R., Kamakaka, R. T. and Kadonaga, J. T. (1999) The RCAF complex mediates chromatin assembly during DNA replication and repair. Nature 402, 555–560.
185 Sharp, J. A., Fouts, E. T., Krawitz, D. C. and Kaufman, P. D. (2001) Yeast histone deposition protein Asf1p requires Hir proteins and PCNA for heterochromatic silencing. Curr. Biol. 11, 463–473.
186 Sarraf, S. A. and Stancheva, I. (2004) Methyl-CpG binding protein MBD1 couples histone H3 methylation at lysine 9 by SETDB1 to DNA replication and chromatin assembly. Mol Cell 15, 595–605.
187 Zhang, Z., Shibahara, K. and Stillman, B. (2000) PCNA connects DNA replication to epigenetic inheritance in yeast. Nature 408, 221–225.
188 Monson, E. K., de Bruin, D. and Zakian, V. A. (1997) The yeast Cac1 protein is required for the stable inheritance of transcriptionally repressed chromatin at telomeres. Proc. Natl. Acad. Sci. USA 94, 13081–13086.
189 Shibahara, K., Verreault, A. and Stillman, B. (2000) The N-terminal domains of histones H3 and H4 are not necessary for chromatin assembly factor-1-mediated nucleosome assembly onto replicated DNA *in vitro.* Proc. Natl. Acad. Sci. USA 97, 7766–7771.
190 Halford, S., Wadey, R., Roberts, C., Daw, S. C., M., Whiting, J. A., O'Donnell, H., Dunham, I., Bentley, D., Lindsay, E., Baldini, A., Francis, F., Lehrach, H. et al. (1993) Isolation of a putative transcriptional regulator from the region of 22q11 deleted in DiGeorge syndrome, Shprintzen syndrome and familial congenital heart disease. Hum. Mol. Genet. 2, 2099–2107.
191 Osley, M. A. and Lycan, D. (1987) *trans*-acting regulatory mutations that alter transcription of *Saccharomyces cerevisiae* histone genes. Mol. Cell. Biol. 7, 4204–4210.
192 Sherwood, P. W. and Osley, M. A. (1991) Histone regulatory (*hir*) mutations suppress δ insertion alleles in *Saccharomyces cerevisiae.* Genetics 128, 729–738.
193 Sherwood, P. W., Tsang, S. V.-M. and Osley, M. A. (1993) Characterization of *HIR1* and *HIR2*, two genes required for regulation of histone gene transcription in *Saccharomyces cerevisiae.* Mol. Cell. Biol. 13, 28–38.
194 Lamour, V., Lécluse, Y., Desmaze, C., Spector, M., Bodescot, M., Aurias, A., Osley, M. A. and Lipinski, M. (1995) A human homolog of the *S. cerevisiae HIR1* and *HIR2* transcriptional repressors cloned from the DiGeorge syndrome critical region. Hum. Mol. Genet. 4, 791–799.
195 Ray-Gallet, D., Quivy, J. P., Scamps, C., Martini, E. M.-D., Lipinski, M. and Almouzni, G. (2002) HIRA is critical for a nucleosome assembly pathway independent of DNA synthesis. Mol. Cell 9, 1091–1100.
196 Lipinski, M., Lorain, S., Monier-Gavelle, F., Brendel, C., Scamps, C. and Lécluse, Y. (1998) HIRA, the product of a major gene candidate for chromosome 22q developmental disorders, is a nuclear protein interacting with core histones and histone-binding proteins. Eur. J. Hum. Genet. 6, 36.
197 Lorain, S., Quivy, J. P., Monier-Gavelle, F., Scamps, C., Lécluse, Y., Almouzni, G. and Lipinski, M. (1998) Core histones and HIRIP3, a novel histone-binding protein, directly interact with WD repeat protein HIRA. Mol. Cell. Biol. 18, 5546–5556.
198 Magnaghi, P., Roberts, C., Lorain, S., Lipinski, M. and Scambler, P. J. (1998) HIRA, a mammalian homologue of *Saccharomyces cerevisiae* transcriptional co-repressors, interacts with Pax3. Nat. Genet. 20, 74–77.
199 Spector, M. S., Raff, A., DeSilva, H., Lee, K. and Osley, M. A. (1997) Hir1p and Hir2p function as transcriptional corepressors to regulate histone gene transcription in the *Saccharomyces cerevisiae* cell cycle. Mol. Cell. Biol. 17, 545–552.
200 DeSilva, H., Lee, K. and Osley, M. A. (1998) Functional dissection of yeast Hir1p, a WD repeat-containing transcriptional corepressor. Genetics 148, 657–667.
201 Xu, H. X., Kim, U. J., Schuster, T. and Grunstein, M. (1992) Identification of a new set of cell cycle-regulatory genes that regulate S-phase transcription of histone genes in *Saccharomyces cerevisiae.* Mol. Cell. Biol. 12, 5249–5259.
202 Prochasson, P., Florens, L., Swanson, S. K., Washburn, M. P. and Workman, J. L. (2005) The HIR corepressor complex binds to nucleosomes generating a distinct protein/DNA complex resistant to remodeling by SWI/SNF. Genes Dev. 19, 2534–2539.
203 Green, E. M., Antczak, A. J., Bailey, A. O., Franco, A. A., Wu, K. J., Yates, J. R. and Kaufman, P. D. (2005) Replication-independent histone deposition by the HIR complex and Asf1. Curr. Biol. 15, 2044–2049.
204 Cross, S. L. and Smith, M. M. (1988) Comparison of the structure and cell cycle expression of mRNAs encoded by two histone H3-H4 loci in *Saccharomyces cerevisiae.* Mol. Cell. Biol. 8, 945–954.
205 Hereford, L., Bromley, S. and Osley, M. A. (1982) Periodic transcription of yeast histone genes. Cell 30, 305–310.
206 Hereford, L. M., Osley, M. A., Ludwig, T. R. and McLaughlin, C. S. (1981) Cell-cycle regulation of yeast histone mRNA. Cell 24, 367–375.
207 White, J. H., M., Green, S. R., Barker, D. G., Dumas, L. B. and Johnston, L. H. (1987) The *CDC8* transcript is cell cycle regulated in yeast and is expressed coordinately with *CDC9* and *CDC21* at a point preceding histone transcription. Exp. Cell Res. 171, 223–231.
208 Dimova, D., Nackerdien, Z., Furgeson, S., Eguchi, S. and Osley, M. A. (1999) A role for transcriptional repressors in targeting the yeast Swi/Snf complex. Mol. Cell 4, 75–83.
209 Hall, C., Nelson, D. M., Ye, X., Baker, K., DeCaprio, J. A., Seeholzer, S., Lipinski, M. and Adams, P. D. (2001) HIRA, the

human homologue of yeast Hir1p and Hir2p, is a novel cyclin-cdk2 substrate whose expression blocks S-phase progression. Mol. Cell. Biol. 21, 1854–1865.
210 De Lucia, F., Lorain, S., Scamps, C., Galisson, F., MacHold, J. and Lipinski, M. (2001) Subnuclear localization and mitotic phosphorylation of HIRA, the human homologue of *Saccharomyces cerevisiae* transcriptional regulators Hir1p/Hir2p. Biochem. J. 358, 447–455.
211 Formosa, T., Ruone, S., Adams, M. D., Olsen, A. E., Eriksson, P., Yu, Y., Rhoades, A. R., Kaufman, P. D. and Stillman, D. J. (2002) Defects in *SPT16* or *POB3* (yFACT) in *Saccharomyces cerevisiae* cause dependence on the Hir/Hpc pathway: Polymerase passage may degrade chromatin structure. Genetics 162, 1557–1571.
212 Ahmad, A., Takami, Y. and Nakayama, T. (2003) WD dipeptide motifs and LXXLL motif of chicken HIRA are necessary for transcription repression and the latter motif is essential for interaction with histone deacetylase-2 *in vivo*. Biochem. Biophys. Res. Commun. 312, 1266–1272.
213 Sutton, A., Bucaria, J., Osley, M. A. and Sternglanz, R. (2001) Yeast ASF1 protein is required for cell cycle regulation of histone gene transcription. Genetics 158, 587–596.
214 Zhang, R., Liu, S. T., Chen, W., Bonner, M., Pehrson, J., Yen, T. J. and Adams, P. D. (2007) HP1 proteins are essential for a dynamic nuclear response that rescues the function of perturbed heterochromatin in primary human cells. Mol. Cell. Biol. 27, 949–962.
215 Blackwell, C., Martin, K. A., Greenall, A., Pidoux, A., Allshire, R. C. and Whitehall, S. K. (2004) The *Schizosaccharomyces pombe* HIRA-Like protein Hip1 is required for the periodic expression of histone genes and contributes to the function of complex centromeres. Mol. Cell. Biol. 24, 4309–4320.
216 Daganzo, S. M., Erzberger, J. P., Lam, W. M., Skordalakes, E., Zhang, R., Franco, A. A., Brill, S. J., Adams, P. D., Berger, J. M. and Kaufman, P. D. (2003) Structure and function of the conserved core of histone deposition protein Asf1. Curr. Biol. 13, 2148–2158.
217 Zhang, R., Poustovoitov, M. V., Ye, X., Santos, H. A., Chen, W., Daganzo, S. M., Erzberger, J. P., Serebriiskii, I. G., Canutescu, A. A., Dunbrack, R. L., Pehrson, J. R., Berger, J. M., Kaufman, P. D. and Adams, P. D. (2005) Formation of MacroH2A-containing senescence-associated heterochromatin foci and senescence driven by ASF1a and HIRA. Dev. Cell 8, 19–30.
218 Schermer, U. J., Korber, P. and Hörz, W. (2005) Histones are incorporated in *trans* during reassembly of the yeast *PHO5* promoter. Mol. Cell 19, 279–285.
219 Loppin, B., Bonnefoy, E., Anselme, C., Laurencon, A., Karr, T. L. and Couble, P. (2005) The histone H3.3 chaperone HIRA is essential for chromatin assembly in the male pronucleus. Nature 437, 1386–1390.
220 van der Heijden, G. W., Dieker, J. W., Derijck, A. A., H. A., Muller, S., Berden, J. H., M., Braat, D. D., M., van der Vlag, J. and de Boer, P. (2005) Asymmetry in histone H3 variants and lysine methylation between paternal and maternal chromatin of the early mouse zygote. Mech. Dev. 122, 1008–1022.
221 Munakata, T., Adachi, N., Yokoyama, N., Kuzuhara, T. and Horikoshi, M. (2000) A human homologue of yeast anti-silencing factor has histone chaperone activity. Genes Cells 5, 221–233.
222 Mousson, F., Lautrette, A., Thuret, J. Y., Agez, M., Courbeyrette, G., Amigues, B., Becker, E., Neumann, J. M., Guerois, R., Mann, C. and Ochsenbein, F. O. (2005) Structural basis for the interaction of Asf1 with histone H3 and its functional implications. Proc. Natl. Acad. Sci. USA 102, 5975–5980.
223 English, C. M., Adkins, M. W., Carson, J. J., Churchill, M. E., A. and Tyler, J. K. (2006) Structural basis for the histone chaperone activity of Asf1. Cell 127, 495–508.
224 Natsume, R., Eitoku, M., Akai, Y., Sano, N., Horikoshi, M. and Senda, T. (2007) Structure and function of the histone chaperone CIA/ASF1 complexed with histones H3 and H4. Nature 446, 338–341.
225 Le, S. Y., Davis, C., Konopka, J. B. and Sternglanz, R. (1997) Two new S-phase-specific genes from *Saccharomyces cerevisiae*. Yeast 13, 1029–1042.
226 Chimura, T., Kuzuhara, T. and Horikoshi, M. (2002) Identification and characterization of CIA/ASF1 as an interactor of bromodomains associated with TFIID. Proc. Natl. Acad. Sci. USA 99, 9334–9339.
227 Padmanabhan, B., Kataoka, K., Umehara, T., Adachi, N., Yokoyama, S. and Horikoshi, M. (2005) Structural similarity between histone chaperone Cia1p/Asf1p and DNA-binding protein NF-κB. J. Biochem. (Tokyo) 138, 821–829.
228 Umehara, T., Chimura, T., Ichikawa, N. and Horikoshi, M. (2002) Polyanionic stretch-deleted histone chaperone cia1/Asf1p is functional both *in vivo* and *in vitro*. Genes Cells 7, 59–73.
229 Silljé, H. H., W. and Nigg, E. A. (2001) Identification of human Asf1 chromatin assembly factors as substrates of Tousled-like kinases. Curr. Biol. 11, 1068–1073.
230 Umehara, T. and Horikoshi, M. (2003) Transcription initiation factor IID-interactive histone chaperone CIA-II implicated in mammalian spermatogenesis. J. Biol. Chem. 278, 35660–35667.
231 Zabaronick, S. R. and Tyler, J. K. (2005) The histone chaperone anti-silencing function 1 is a global regulator of transcription independent of passage through S phase. Mol. Cell. Biol. 25, 652–660.
232 Adkins, M. W., Howar, S. R. and Tyler, J. K. (2004) Chromatin disassembly mediated by the histone chaperone Asf1 is essential for transcriptional activation of the yeast *PHO5* and *PHO8* genes. Mol. Cell 14, 657–666.
233 Schwabish, M. A. and Struhl, K. (2006) Asf1 mediates histone eviction and deposition during elongation by RNA polymerase II. Mol. Cell 22, 415–422.
234 Adkins, M. W., Williams, S. K., Linger, J. and Tyler, J. K. (2007) Chromatin disassembly from the *PHO5* promoter is essential for the recruitment of the general transcription machinery and coactivators. Mol. Cell. Biol. 27, 6372–6382.
235 Franco, A. A., Lam, W. M., Burgers, P. M. and Kaufman, P. D. (2005) Histone deposition protein Asf1 maintains DNA replisome integrity and interacts with replication factor C. Genes Dev. 19, 1365–1375.
236 Schulz, L. L. and Tyler, J. K. (2006) The histone chaperone ASF1 localizes to active DNA replication forks to mediate efficient DNA replication. FASEB J. 20, 488–490.
237 Emili, A., Schieltz, D. W., Yates, J. R. and Hartwell, L. H. (2001) Dynamic interaction of DNA damage checkpoint protein Rad53 with chromatin assembly factor Asf1. Mol. Cell 7, 13–20.
238 Hu, F. H., Alcasabas, A. A. and Elledge, S. J. (2001) Asf1 links Rad53 to control of chromatin assembly. Genes Dev. 15, 1061–1066.
239 Lee, S. J., Schwartz, M. F., Duong, J. K. and Stern, D. F. (2003) Rad53 phosphorylation site clusters are important for Rad53 regulation and signaling. Mol. Cell. Biol. 23, 6300–6314.
240 Ramey, C. J., Howar, S., Adkins, M., Linger, J., Spicer, J. and Tyler, J. K. (2004) Activation of the DNA damage checkpoint in yeast lacking the histone chaperone anti-silencing function 1. Mol. Cell. Biol. 24, 10313–10327.
241 Prado, F., Cortés-Ledesma, F. and Aguilera, A. (2004) The absence of the yeast chromatin assembly factor Asf1 increases genomic instability and sister chromatid exchange. EMBO Rep. 5, 497–502.
242 Osada, S., Sutton, A., Muster, N., Brown, C. E., Yates, J. R., Sternglanz, R. and Workman, J. L. (2001) The yeast SAS (something about silencing) protein complex contains a MYST-type putative acetyltransferase and functions with chromatin assembly factor ASF1. Genes Dev. 15, 3155–3168.
243 Meijsing, S. H. and Ehrenhofer-Murray, A. E. (2001) The silencing complex SAS-I links histone acetylation to the

assembly of repressed chromatin by CAF-I and Asf1 in *Saccharomyces cerevisiae.* Genes Dev. 15, 3169–3182.
244 Moshkin, Y. M., Armstrong, J. A., Maeda, R. K., Tamkun, J. W., Verrijzer, P., Kennison, J. A. and Karch, F. (2002) Histone chaperone ASF1 cooperates with the Brahma chromatin-remodelling machinery. Genes Dev. 16, 2621–2626.
245 Carrera, P., Moshkin, Y. M., Grönke, S., Silljé, H. H., W., Nigg, E. A., Jäckle, H. and Karch, F. (2003) Tousled-like kinase functions with the chromatin assembly pathway regulating nuclear divisions. Genes Dev. 17, 2578–2590.
246 Ehsan, H., Reichheld, J. P., Durfee, T. and Roe, J. L. (2004) TOUSLED kinase activity oscillates during the cell cycle and interacts with chromatin regulators. Plant Physiol. 134, 1488–1499.
247 Yamaki, M., Umehara, T., Chimura, T. and Horikoshi, M. (2001) Cell death with predominant apoptotic features in *Saccharomyces cerevisiae* mediated by deletion of the histone chaperone ASF1/CIA1. Genes Cells 6, 1043–1054.
248 Ye, X., Zerlanko, B., Zhang, R., Somaiah, N., Lipinski, M., Salomoni, P. and Adams, P. D. (2007) Definition of pRB- and p53-dependent and -independent steps in HIRA/ASF1a-mediated formation of senescence-associated heterochromatin foci. Mol. Cell. Biol. 27, 2452–2465.
249 English, C. M., Maluf, N. K., Tripet, B., Churchill, M. E., A. and Tyler, J. K. (2005) ASF1 binds to a heterodimer of histones H3 and H4: A two-step mechanism for the assembly of the H3-H4 heterotetramer on DNA. Biochemistry 44, 13673–13682.
250 Antczak, A. J., Tsubota, T., Kaufman, P. D. and Berger, J. M. (2006) Structure of the yeast histone H3-ASF1 interaction: Implications for chaperone mechanism, species-specific interactions, and epigenetics. BMC Struct. Biol. 6, 26.
251 Agez, M., Chen, J., Guerois, R., van Heijenoort, C., Thuret, J. Y., Mann, C. and Ochsenbein, F. (2007) Structure of the histone chaperone Asf1 bound to the histone H3 C-terminal helix and functional insights. Structure 15, 191–199.
252 Groth, A., Ray-Gallet, D., Quivy, J. P., Lukas, J., Bartek, J. and Almouzni, G. (2005) Human Asf1 regulates the flow of S phase histones during replicational stress. Mol. Cell 17, 301–311.
253 Sutton, A., Shia, W. J., Band, D., Kaufman, P. D., Osada, S., Workman, J. L. and Sternglanz, R. (2003) Sas4 and Sas5 are required for the histone acetyltransferase activity of Sas2 in the SAS complex. J. Biol. Chem. 278, 16887–16892.
254 Driscoll, R., Hudson, A. and Jackson, S. P. (2007) Yeast Rtt109 promotes genome stability by acetylating histone H3 on lysine 56. Science 315, 649–652.
255 Han, J., Zhou, H., Horazdovsky, B., Zhang, K., Xu, R. M. and Zhang, Z. (2007) Rtt109 acetylates histone H3 lysine 56 and functions in DNA replication. Science 315, 653–655.
256 Collins, S. R., Miller, K. M., Maas, N. L., Roguev, A., Fillingham, J., Chu, C. S., Schuldiner, M., Gebbia, M., Recht, J., Shales, M., Ding, H., Xu, H. et al. (2007) Functional dissection of protein complexes involved in yeast chromosome biology using a genetic interaction map. Nature 446, 806–810.
257 Ferreira, H., Somers, J., Webster, R., Flaus, A. and Owen-Hughes, T. (2007) Histone tails and the H3 αN helix regulate nucleosome mobility and stability. Mol. Cell. Biol. 27, 4037–4048.
258 Ray-Gallet, D., Quivy, J. P., Silljé, H. W., W., Nigg, E. A. and Almouzni, G. (2007) The histone chaperone Asf1 is dispensable for direct *de novo* histone deposition in *Xenopus* egg extracts. Chromosoma 116, 487–496.
259 Kuzuhara, T. and Horikoshi, M. (2004) A nuclear FK506-binding protein is a histone chaperone regulating rDNA silencing. Nat. Struct. Mol. Biol. 11, 275–283.
260 Shan, X., Xue, Z. and Mélèse, T. (1994) Yeast *NPI46* encodes a novel prolyl *cis-trans* isomerase that is located in the nucleolus. J. Cell Biol. 126, 853–862.
261 Benton, B. M., Zang, J. H. and Thorner, J. (1994) A novel FK506- and rapamycin-binding protein (FPR3 gene product) in the yeast *Saccharomyces cerevisiae* is a proline rotamase localized to the nucleolus. J. Cell Biol. 127, 623–639.
262 Manning-Krieg, U. C., Henríquez, R., Cammas, F., Graff, P., Gavériaux, S. and Movva, N. R. (1994) Purification of FKBP-70, a novel immunophilin from *Saccharomyces cerevisiae*, and cloning of its structural gene, *FPR3.* FEBS Lett. 352, 98–103.
263 Dolinski, K., Muir, S., Cardenas, M. and Heitman, J. (1997) All cyclophilins and FK506 binding proteins are, individually and collectively, dispensable for viability in *Saccharomyces cerevisiae.* Proc. Natl. Acad. Sci. USA 94, 13093–13098.
264 Himukai, R., Kuzuhara, T. and Horikoshi, M. (1999) Relationship between the subcellular localization and structures of catalytic domains of FKBP-type PPIases. J. Biochem. (Tokyo) 126, 879–888.
265 Angelov, D., Bondarenko, V. A., Almagro, S., Menoni, H., Mongélard, F., Hans, F., Mietton, F., Studitsky, V. M., Hamiche, A., Dimitrov, S. and Bouvet, P. (2006) Nucleolin is a histone chaperone with FACT-like activity and assists remodeling of nucleosomes. EMBO J. 25, 1669–1679.
266 Shou, W., Sakamoto, K. M., Keener, J., Morimoto, K. W., Traverso, E. E., Azzam, R., Hoppe, G. J., Feldman, R. M., R., DeModena, J., Moazed, D., Charbonneau, H., Nomura, M. and Deshaies, R. J. (2001) Net1 stimulates RNA polymerase I transcription and regulates nucleolar structure independently of controlling mitotic exit. Mol. Cell 8, 45–55.
267 Xiao, H., Jackson, V. and Lei, M. (2006) The FK506-binding protein, Fpr4, is an acidic histone chaperone. FEBS Lett. 580, 4357–4364.
268 Nelson, C. J., Santos-Rosa, H. and Kouzarides, T. (2006) Proline isomerization of histone H3 regulates lysine methylation and gene expression. Cell 126, 905–916.
269 Luk, E., Vu, N. D., Patteson, K., Mizuguchi, G., Wu, W. H., Ranjan, A., Backus, J., Sen, S., Lewis, M., Bai, Y. W. and Wu, C. (2007) Chz1, a nuclear chaperone for histone H2AZ. Mol. Cell 25, 357–368.
270 Wilson, L. K., Benton, B. M., Zhou, S., Thorner, J. and Martin, G. S. (1995) The yeast immunophilin Fpr3 is a physiological substrate of the tyrosine-specific phosphoprotein phosphatase Ptp1. J. Biol. Chem. 270, 25185–25193.
271 Wilson, L. K., Dhillon, N., Thorner, J. and Martin, G. S. (1997) Casein kinase II catalyzes tyrosine phosphorylation of the yeast nucleolar immunophilin Fpr3. J. Biol. Chem. 272, 12961–12967.
272 Marchetta, M., Gamberi, T., Sarno, S., Magherini, F., Raugei, G., Camici, G., Pinna, L. A. and Modesti, A. (2004) Expression of the Stp1 LMW-PTP and inhibition of protein CK2 display a cooperative effect on immunophilin Fpr3 tyrosine phosphorylation and *Saccharomyces cerevisiae* growth. Cell. Mol. Life Sci. 61, 1176–1184.
273 Hochwagen, A., Tham, W. H., Brar, G. A. and Amon, A. (2005) The FK506 binding protein Fpr3 counteracts protein phosphatase 1 to maintain meiotic recombination checkpoint activity. Cell 122, 861–873.
274 Utsugi, T., Toh-e, A. and Kikuchi, Y. (1995) A high dose of the *STM1* gene suppresses the temperature sensitivity of the *tom1* and *htr1* mutants in *Saccharomyces cerevisiae.* Biochim. Biophys. Acta 1263, 285–288.
275 Utsugi, T., Hirata, A., Sekiguchi, Y., Sasaki, T., Toh-e, A. and Kikuchi, Y. (1999) Yeast *tom1* mutant exhibits pleiotropic defects in nuclear division, maintenance of nuclear structure and nucleocytoplasmic transport at high temperatures. Gene 234, 285–295.
276 Davey, M., Hannam, C., Wong, C. and Brandl, C. J. (2000) The yeast peptidyl proline isomerases FPR3 and FPR4, in high copy numbers, suppress defects resulting from the absence of the E3 ubiquitin ligase TOM1. Mol. Gen. Genet. 263, 520–526.
277 Aronheim, A., Zandi, E., Hennemann, H., Elledge, S. J. and Karin, M. (1997) Isolation of an AP-1 repressor by a novel

method for detecting protein-protein interactions. Mol. Cell. Biol. 17, 3094–3102.
278 Jin, C., Ugai, H., Song, J., Murata, T., Nili, F., Sun, K., Horikoshi, M. and Yokoyama, K. K. (2001) Identification of mouse Jun dimerization protein 2 as a novel repressor of ATF-2. FEBS Lett. 489, 34–41.
279 Jin, C., Kato, K., Chimura, T., Yamasaki, T., Nakade, K., Murata, T., Li, H., Pan, J., Zhao, M., Sun, K., Chiu, R., Ito, T., Nagata, K., Horikoshi, M. and Yokoyama, K. K. (2006) Regulation of histone acetylation and nucleosome assembly by transcription factor JDP2. Nat. Struct. Mol. Biol. 13, 331–338.
280 Katz, S., Heinrich, R. and Aronheim, A. (2001) The AP-1 repressor, JDP2, is a bona fide substrate for the c-Jun N-terminal kinase. FEBS Lett. 506, 196–200.
281 Katz, S. and Aronheim, A. (2002) Differential targeting of the stress mitogen-activated protein kinases to the c-Jun dimerization protein 2. Biochem. J. 368, 939–945.
282 Wardell, S. E., Boonyaratanakornkit, V., Adelman, J. S., Aronheim, A. and Edwards, D. P. (2002) Jun dimerization protein 2 functions as a progesterone receptor N-terminal domain coactivator. Mol. Cell. Biol. 22, 5451–5466.
283 Broder, Y. C., Katz, S. and Aronheim, A. (1998) The Ras recruitment system, a novel approach to the study of protein-protein interactions. Curr. Biol. 8, 1121–1124.
284 Jin, C., Li, H., Murata, T., Sun, K., Horikoshi, M., Chiu, R. and Yokoyama, K. K. (2002) JDP2, a repressor of AP-1, recruits a histone deacetylase 3 complex to inhibit the retinoic acid-induced differentiation of F9 cells. Mol. Cell. Biol. 22, 4815–4826.
285 Wardell, S. E., Kwok, S. C., Sherman, L., Hodges, R. S. and Edwards, D. P. (2005) Regulation of the amino-terminal transcription activation domain of progesterone receptor by a cofactor-induced protein folding mechanism. Mol. Cell. Biol. 25, 8792–8808.
286 Ostrovsky, O., Bengal, E. and Aronheim, A. (2002) Induction of terminal differentiation by the c-Jun dimerization protein JDP2 in C2 myoblasts and rhabdomyosarcoma cells. J. Biol. Chem. 277, 40043–40054.
287 Kawaida, R., Ohtsuka, T., Okutsu, J., Takahashi, T., Kadono, Y., Oda, H., Hikita, A., Nakamura, K., Tanaka, S. and Furukawa, H. (2003) Jun dimerization protein 2 (JDP2), a member of the AP-1 family of transcription factor, mediates osteoclast differentiation induced by RANKL. J. Exp. Med. 197, 1029–1035.
288 Heinrich, R., Livne, E., Ben-Izhak, O. and Aronheim, A. (2004) The c-Jun dimerization protein 2 inhibits cell transformation and acts as a tumor suppressor gene. J. Biol. Chem. 279, 5708–5715.
289 Davies, M., Robinson, M., Smith, E., Huntley, S., Prime, S. and Paterson, I. (2005) Induction of an epithelial to mesenchymal transition in human immortal and malignant keratinocytes by TGF-β1 involves MAPK, Smad and AP-1 signalling pathways. J. Cell. Biochem. 95, 918–931.
290 Piu, F., Aronheim, A., Katz, S. and Karin, M. (2001) AP-1 repressor protein JDP-2: Inhibition of UV-mediated apoptosis through p53 down-regulation. Mol. Cell. Biol. 21, 3012–3024.
291 Lerdrup, M., Holmberg, C., Dietrich, N., Shaulian, E., Herdegen, T., Jäättelä, M. and Kallunki, T. (2005) Depletion of the AP-1 repressor JDP2 induces cell death similar to apoptosis. Biochim. Biophys. Acta 1745, 29–37.
292 Crevel, G. and Cotterill, S. (1995) DF 31, a sperm decondensation factor from *Drosophila melanogaster:* Purification and characterization. EMBO J. 14, 1711–1717.
293 Crevel, G., Huikeshoven, H. and Cotterill, S. (2001) Df31 is a novel nuclear protein involved in chromatin structure in *Drosophila melanogaster.* J. Cell Sci. 114, 37–47.
294 Huang, S. B., Zhou, H., Katzmann, D., Hochstrasser, M., Atanasova, E. and Zhang, Z. (2005) Rtt106p is a histone chaperone involved in heterochromatin-mediated silencing. Proc. Natl. Acad. Sci. USA 102, 13410–13415.
295 Huang, S., Zhou, H., Tarara, J. and Zhang, Z. (2007) A novel role for histone chaperones CAF-1 and Rtt106p in heterochromatin silencing. EMBO J. 26, 2274–2283.
296 Belotserkovskaya, R., Oh, S., Bondarenko, V. A., Orphanides, G., Studitsky, V. M. and Reinberg, D. (2003) FACT facilitates transcription-dependent nucleosome alteration. Science 301, 1090–1093.
297 Clark-Adams, C. D., Norris, D., Osley, M. A., Fassler, J. S. and Winston, F. (1988) Changes in histone gene dosage alter transcription in yeast. Genes Dev. 2, 150–159.
298 Prendergast, J. A., Murray, L. E., Rowley, A., Carruthers, D. R., Singer, R. A. and Johnston, G. C. (1990) Size selection identifies new genes that regulate *Saccharomyces cerevisiae* cell proliferation. Genetics 124, 81–90.
299 Malone, E. A., Clark, C. D., Chiang, A. and Winston, F. (1991) Mutations in *SPT16/CDC68* suppress *cis-* and *trans-*acting mutations that affect promoter function in *Saccharomyces cerevisiae.* Mol. Cell. Biol. 11, 5710–5717.
300 Rowley, A., Singer, R. A. and Johnston, G. C. (1991) *CDC68*, a yeast gene that affects regulation of cell proliferation and transcription, encodes a protein with a highly acidic carboxyl terminus. Mol. Cell. Biol. 11, 5718–5726.
301 Wittmeyer, J. and Formosa, T. (1997) The *Saccharomyces cerevisiae* DNA polymerase α catalytic subunit interacts with Cdc68/Spt16 and with Pob3, a protein similar to an HMG1-like protein. Mol. Cell. Biol. 17, 4178–4190.
302 Orphanides, G., Wu, W. H., Lane, W. S., Hampsey, M. and Reinberg, D. (1999) The chromatin-specific transcription elongation factor FACT comprises human SPT16 and SSRP1 proteins. Nature 400, 284–288.
303 Orphanides, G., LeRoy, G., Chang, C. H., Luse, D. S. and Reinberg, D. (1998) FACT, a factor that facilitates transcript elongation through nucleosomes. Cell 92, 105–116.
304 Okuhara, K., Ohta, K., Seo, H., Shioda, M., Yamada, T., Tanaka, Y., Dohmae, N., Seyama, Y., Shibata, T. and Murofushi, H. (1999) A DNA unwinding factor involved in DNA replication in cell-free extracts of *Xenopus* eggs. Curr. Biol. 9, 341–350.
305 Formosa, T. (2003) Changing the DNA landscape: Putting a SPN on chromatin. Curr. Top. Microbiol. Immunol. 274, 171–201.
306 Brewster, N. K., Johnston, G. C. and Singer, R. A. (2001) A bipartite yeast SSRP1 analog comprised of Pob3 and Nhp6 proteins modulates transcription. Mol. Cell. Biol. 21, 3491–3502.
307 Formosa, T., Eriksson, P., Wittmeyer, J., Ginn, J., Yu, Y. and Stillman, D. J. (2001) Spt16-Pob3 and the HMG protein Nhp6 combine to form the nucleosome-binding factor SPN. EMBO J. 20, 3506–3517.
308 Ruone, S., Rhoades, A. R. and Formosa, T. (2003) Multiple Nhp6 molecules are required to recruit Spt16-Pob3 to form yFACT complexes and to reorganize nucleosomes. J. Biol. Chem. 278, 45288–45295.
309 Kang, S. W., Kuzuhara, T. and Horikoshi, M. (2000) Functional interaction of general transcription initiation factor TFIIE with general chromatin factor SPT16/CDC68. Genes Cells 5, 251–263.
310 Biswas, D., Yu, Y., Prall, M., Formosa, T. and Stillman, D. J. (2005) The yeast FACT complex has a role in transcriptional initiation. Mol. Cell. Biol. 25, 5812–5822.
311 Shimojima, T., Okada, M., Nakayama, T., Ueda, H., Okawa, K., Iwamatsu, A., Handa, H. and Hirose, S. (2003) *Drosophila* FACT contributes to Hox gene expression through physical and functional interactions with GAGA factor. Genes Dev. 17, 1605–1616.
312 Nakayama, T., Nishioka, K., Dong, Y. X., Shimojima, T. and Hirose, S. (2007) *Drosophila* GAGA factor directs histone H3.3 replacement that prevents the heterochromatin spreading. Genes Dev. 21, 552–561.
313 Mason, P. B. and Struhl, K. (2003) The FACT complex travels with elongating RNA polymerase II and is important for the

fidelity of transcriptional initiation *in vivo*. Mol. Cell. Biol. 23, 8323–8333.
314 Duroux, M., Houben, A., Růžička, K., Friml, J. and Grasser, K. D. (2004) The chromatin remodelling complex FACT associates with actively transcribed regions of the *Arabidopsis* genome. Plant J. 40, 660–671.
315 Pavri, R., Zhu, B., Li, G., Trojer, P., Mandal, S., Shilatifard, A. and Reinberg, D. (2006) Histone H2B monoubiquitination functions cooperatively with FACT to regulate elongation by RNA polymerase II. Cell 125, 703–717.
316 Tan, B. C., M., Chien, C. T., Hirose, S. and Lee, S. C. (2006) Functional cooperation between FACT and MCM helicase facilitates initiation of chromatin DNA replication. EMBO J. 25, 3975–3985.
317 Keller, D. M., Zeng, X., Wang, Y., Zhang, Q. H., Kapoor, M., Shu, H., Goodman, R., Lozano, G., Zhao, Y. and Lu, H. (2001) A DNA damage-induced p53 serine 392 kinase complex contains CK2, hSpt16, and SSRP1. Mol. Cell 7, 283–292.
318 Keller, D. M. and Lu, H. (2002) p53 serine 392 phosphorylation increases after UV through induction of the assembly of the CK2·hSPT16·SSRP1 complex. J. Biol. Chem. 277, 50206–50213.
319 Ito, T., Bulger, M., Pazin, M. J., Kobayashi, R. and Kadonaga, J. T. (1997) ACF, an ISWI-containing and ATP-utilizing chromatin assembly and remodeling factor. Cell 90, 145–155.
320 Loyola, A., LeRoy, G., Wang, Y. H. and Reinberg, D. (2001) Reconstitution of recombinant chromatin establishes a requirement for histone-tail modifications during chromatin assembly and transcription. Genes Dev. 15, 2837–2851.
321 Ishimi, Y., Komamura-Kohno, Y., Arai, K. and Masai, H. (2001) Biochemical activities associated with mouse Mcm2 protein. J. Biol. Chem. 276, 42744–42752.
322 Gasser, R., Koller, T. and Sogo, J. M. (1996) The stability of nucleosomes at the replication fork. J. Mol. Biol. 258, 224–239.
323 Sharma, R., Nakamura, A., Takahashi, R., Nakamoto, H. and Goto, S. (2006) Carbonyl modification in rat liver histones: Decrease with age and increase by dietary restriction. Free Radic. Biol. Med. 40, 1179–1184.
324 Haas, A., Reback, P. M., Pratt, G. and Rechsteiner, M. (1990) Ubiquitin-mediated degradation of histone H3 does not require the substrate-binding ubiquitin protein ligase, *E3*, or attachment of polyubiquitin chains. J. Biol. Chem. 265, 21664–21669.
325 Watson, J. D. and Crick, F. H. C. (1953) Genetical implications of the structure of deoxyribonucleic acid. Nature 171, 964–967.
326 Meselson, M. and Stahl, F. W. (1958) The replication of DNA in *Escherichia coli*. Proc. Natl. Acad. Sci. USA 44, 671–682.
327 Polo, S. E. and Almouzni, G. (2006) Chromatin assembly: A basic recipe with various flavours. Curr. Opin. Genet. Dev. 16, 104–111.
328 Endoh, M., Zhu, W., Hasegawa, J., Watanabe, H., Kim, D. K., Aida, M., Inukai, N., Narita, T., Yamada, T., Furuya, A., Sato, H., Yamaguchi, Y. et al. (2004) Human Spt6 stimulates transcription elongation by RNA polymerase II *in vitro*. Mol. Cell. Biol. 24, 3324–3336.
329 Lorain, S., Lécluse, Y., Scamps, C., Mattéi, M. G. and Lipinski, M. (2001) Identification of human and mouse HIRA-interacting protein-5 (HIRIP5), two mammalian representatives in a family of phylogenetically conserved proteins with a role in the biogenesis of Fe/S proteins. Biochim. Biophys. Acta 1517, 376–383.